Norwegian University of Life Sciences

Master's Thesis 2020 60 ECTS

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Habitat and Foraging Ecology of Two Cryptic Bat Species 59° North; *Myotis mystacinus and M. brandtii*

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Abstract

The Brandts' bat (*Myotis* brandtii) and the whiskered bat (*Myotis mystacinus*) are cryptic species from distinct taxonomic clades whose ranges frequently overlap. What, if any, resource partitioning that exists between them is not well understood, especially at the northern extent of their range. The aim of this study is to compare the foraging ecology, roost ecology and diet of *M. brandtii* and *M. mystacinus* during the summer in southeastern Norway.

Bats were captured using mist nets and adult females were radio-tagged. Tagged individuals were tracked for a week on average, during which time the entire period of foraging every night and roost activity were monitored. LiDAR (light detection and ranging) data describing habitat structure in observed foraging locations, and an equal number of random locations within each bat's home range, were used to build resource selection functions (RSFs) to evaluate differences in foraging habitat use between the two species. Feces from captured bats were analyzed using molecular genetic analysis to verify in-hand identification of these cryptic species in addition to dietary analyses.

I found that forests with varied canopy height were important foraging locations for both species but that *M. mystacinus* was predicted to use more diverse vegetation structure than *M. brandtii,* reinforcing previous research which has proposed that *M. brandtii* is more specialized to mature forests while *M. mystacinus* is more generalized in habitat selection. The home range of *M. brandtii* was over 3 times the size of *M. mystacinus,* further suggesting that *M. brandtii* is willing to travel farther distances to reach specific habitat than *M. mystacinus* that is adapted to using a variety of foraging habitat. Both species utilized similar roosts, with colonies only being found in the roofs of houses.

This is the first study to compare the foraging ecology of *M. brandtii* and *M. mystacinus* using 3-dimensional continuous descriptions of habitat. Furthermore, it is one of few studies that has accomplished homing in on radio-tagged bats to collect precise foraging locations. The findings here can provide insights into monitoring techniques that can be applied to studying bats at northern latitudes as well as for studying resource selection of bats globally.

Acknowledgements

I am deeply grateful to Jeroen van der Kooij for sharing his passion and knowledge of wildlife with me; for inspiring me to pursue being bat biologist in Norway, then becoming a patient and caring mentor as the pursuit became a reality. Furthermore, I am fortunate to have Katrine Eldegard as a main supervisor. Katrine has challenged and supported me and invested in my personal growth from the moment I began working with her. I am profoundly grateful for her encouragement and her instruction.

A statistically significant thank you is in order for Richard Bischof in helping to transform me from a field working, data collection junky to someone who now actually enjoys exploring model outputs in R Studio. He in addition to Mahdieh Tourani, have been huge supports in helping me develop my statistical skills and widening my horizons as a wildlife researcher. Thank you, Richard, and Mahdieh.

Thank you to Clare Stawski for your support during the 2018 field season and for your help introducing me to the world of genetics research. I am extremely grateful to Dr. Steven Smith and the genetics lab team at the Konrad Lorenz Institute of Ethology in Vienna for their partnership in analyzing the bat feces for this thesis as well as their generous hospitality in hosting me during my stay. *Danke schön,* Franz Hölzl, for your mentorship and for sharing your zeal for genetics with me.

Thank you to Rune Sørås for your leadership during the 2018 field season as well as your continued support. *Tusen takk* to Karoline Manneråk Birkeland for working so diligently to maintain order as well as “kos” during the field season and getting me settled in my new home. Thank you to Joakim Siljedal and Kristian Kristiansen for your hard work in collecting this data and for keeping spirits high when it mattered most. Thank you to Håvard Angell Hald and Karoline Hansen Skåra for going above and beyond to help in data collection as well as for your wonderful company.

Thank you to research professor Hans Ole Ørka at MINA for preparing the raster stacks of LiDAR variables and your continued assistance in processing this data.

I am greatly appreciative to Goniela Iskali, Larisa Bishop-Boros and Ben Hale for continuing to help me grow as a biologist from afar. A warm thank you to Dr. Vicky Merestsky for helping me to escape windmill flowchart paralysis.

I am endlessly grateful to my parents, Åse and Jim McKay, as well as my sister, Steffi Jo Buie, for supporting me every inch of the way in all my endeavours.

I have been well endowed with the support and care of many friends and colleagues who all deserve novel length descriptions of my admiration. I will save those for later and resolve to say that I would not have survived the last two years without Viktorija and Jarle. Thank you for keeping me well fed and out of trouble.

I am of course, emphatically grateful to my dog. Illiterate though he may be, he has been instrumental in my journey as a biologist and in keeping me true to my goals. Thanks, Pach.

Preface

In order to address some of the more pressing gaps in knowledge regarding bat ecology in Norway, a collaboration between The Norwegian University of Life Sciences and the Norwegian Zoological Society was formed under the name SCANDBAT in 2016. The primary goals of the SCANDBAT project have been to identify important bat habitat and develop monitoring techniques for bats in Norway throughout their annual cycle by studying maternity colonies, foraging habitat, diet and hibernacula sites. This thesis was designed and implemented as a SCANDBAT research initiative.

In 1993, Norway joined The Agreement of the Conservation of Populations of European Bats (EUROBATS). This agreement primarily functions to protect bats throughout Europe by unifying countries in similar goals of conservation of bats through legislation and education. All bat species in Norway are protected through the Wildlife Act (Lov om viltet, 1981) and the national biodiversity act (Lov om forvaltning av naturens mangfold (naturmangfoldloven), 2009). These agreements propose that the Norwegian management authorities should support efforts to monitor and carry out research on bats in order to make informed decisions regarding wildlife management.

Ethics Statement

All wildlife included in this study were treated with the utmost care. Capturing and handling of wildlife was all in accordance with permitted authorizations from the Norwegian Environment Agency (Miljødirektoratet) and the Norwegian Food Safety Authority (Mattilsynet).

1 Introduction

Bats play an integral part of maintaining the health and diversity of Norway's ecosystems. The 13 species that can be found in the country make up over 20% of terrestrial Norwegian mammal life (Isaksen et al. 2009, Wiig et al. 2015, Størkersen et al. 2018). Even though bats are rarely exhibited amongst other prominent Norwegian wildlife, they are present in a large array of ecosystems across the country. Daubentons' bats (*Myotis daubentonii)* can be found foraging along Akerselva in the heart of Oslo while Northern bats (*Eptesicus nilssonii)* raise their pups in the midnight sun of Troms county. Whiskered bats (*Myotis mystacinus)* join Norwegians on their summer cabin trips in Romeriksåsen, roosting inside wall cavities. Brown long-eared bats (*Plecotus auritus)* can be found quietly removing insects along hiking trails in the forests around Trondheim.

All of the species of bats found in Norway are insectivorous and belong to the family *Vespertilionidae*. The largest species known to live in Norway, the common Noctule, *Nyctalus noctula,* rarely gets larger than 30 g. The smallest species, the Soprano Pipistrelle, *Pipistrellus pygmaeus*, can weigh as little as 3 g as an adult. Their small size in addition to their nocturnal and volant nature as well as their propensity to roost in inconspicuous spaces and use vocalizations in a range outside of human hearing, has allowed bats to live in close proximity to people while going unnoticed. This also creates a variety of challenges for bat researchers (Kunz and Fenton 2003, Williams-Guillén et al. 2016).

Even basic monitoring techniques such as banding, and recapturing, is a practice that was not applied in bat research anywhere in the world before the 20th century (Allen 1921). Since then, wildlife research has become far more popular and prioritized in the scientific community (Arroyo et al. 2016). Bat research has subsequently developed a great deal over the last century and benefitted from many technological advancements such as bat acoustic monitoring devices (Griffin et al. 1941, Dijkgraaf 1946) and increasingly sophisticated radio telemetry transmitters (Fleming 1977, Wilkinson and Bradbury 1988). Despite the increase in focus on bat research, bats today are the group of mammals with the largest proportion of data deficient species

(18%) registered under the IUCN Red List (IUCN 2019). There remains a great deal that needs to be learned about bat populations globally in order to make informed decisions regarding their conservation (Frick et al. 2019). These challenges are not unique to bat researchers working in the subarctic, but the environmental conditions found in this part of the world do pose some unique challenges for bats.

The cooler temperatures and shorter night periods experienced by bats at northern latitudes means that these populations have less foraging time than those of conspecifics at lower latitudes, while still needing to meet the same, or potentially higher, critical energy demands associated with pregnancy and lactation during the summer maternity season (Shively et al. 2017). My study took place in Nittedal, Norway where the longest day of the year, the summer solstice, is 18 hours and 53 minutes long (https://www.timeanddate.no). Throughout the summer, there are extended periods of twilight outside of civil sunrise and sunset as well when nocturnal animals may still be vulnerable to increased visibility. These abiotic conditions create an especially restrictive set of circumstances for bat species that are not predisposed to fly in open spaces, such as Brandt's bat, *Myotis brandtii*, and Whiskered bat, *M. mystacinus* (Norberg et al. 1987).

M. brandtii and *M. mystacinus* are cryptic species whose ranges overlap throughout much of Europe. Cryptic species are defined as two or more species that are or have been previously designated as "a single nominal species because they are at least superficially morphologically indistinguishable" (Bickford et al. 2007). Many cryptic species may also be designated as 'sister species' if they exist in the same taxonomic clade or belong to the next most closely related taxonomical grouping (Knowlton 1986). *M. brandtii* and *M. mystacinus* are unique in this way; they are not sister species despite their physical resemblance. *M. brandtii* belongs to the same clade as *Myotis* species found in the Americas whereas *M. mystacinus* is situated in a clade of European *Myotis* species (Ruedi and Mayer 2002, Bickham et al. 2004).

It can be exceptionally difficult to distinguish *M. brandtii* from *M. mystacinus* from each other, even in the hand. Thus, some ecological studies group them together, especially when relying on acoustic monitoring methods (Bach et al. 2004, Wermundsen and Siivonen 2008, Froidevaux et al. 2016). The foraging behavior of these two species

has been studied separately and in comparison, to one another by some previous studies (Taake 1984, Dense and Rahmel 2002, Berge 2007, Buckley et al. 2013, Vesterinen et al. 2018, Roswag et al. 2019), but this has yet to be explored in Norway. There remains a great deal left to be understood regarding what – if any – resource partitioning exists between these two species in terms of foraging selection, roost selection and diet. This is especially true for populations of these species living at the northern latitudes.

Previous research regarding habitat selection of *M. brandtii* and *M. mystacinus* species has found that while both species are frequently found in forests, there is some evidence which suggests that *M. mystacinus* is more likely to forage in cultural landscapes such as near gardens and agricultural lands while *M. brandtii* appears to be more dependent on forested landscapes (Taake 1984, Vaughn et al. 1997a, Zukal et al. 2006, Berge 2007, Buckley et al. 2013, Froidevaux et al. 2016). However, it is challenging to collect data on the location of foraging bats using standard VHF tracking techniques because this typically relies on using triangulations to track bats as they move (often resulting in positions with low precision) or being able to physically follow a bat as it forages (innately challenging and often infeasible). It is nearly impossible to distinguish between *M. brandtii* and *M. mystacinus* based on their foraging echolocations and thus acoustic monitoring is not a viable option for comparing the foraging behavior of these species where their ranges overlap (Vaughan et al. 1997a, Vaughan et al. 1997b, Barataud 2015).

Following the logic of the ecological niche theory (Hutchinson 1957), species that share similarities in geological position and morphology should have different ecological roles and thus I hypothesize that *M. brandtii* and *M. mystacinus* fulfill distinct ecological roles that should be exhibited in their summer roost, foraging and diet selection. The goal of this study was to explore the foraging habitat selection, diet and roost selection of adult female *M. brandtii* and *M. mystacinus* in southeastern Norway during the summer maternity season by addressing the following research questions:

- How does selection of foraging habitat and roosts compare between *M. brandtii* and *M. mystacinus*?
- How does home range size compare between *M. brandtii* and *M. mystacinus*?
- What, if any, differences in diet exist between *M. brandtii* and *M. mystacinus?*

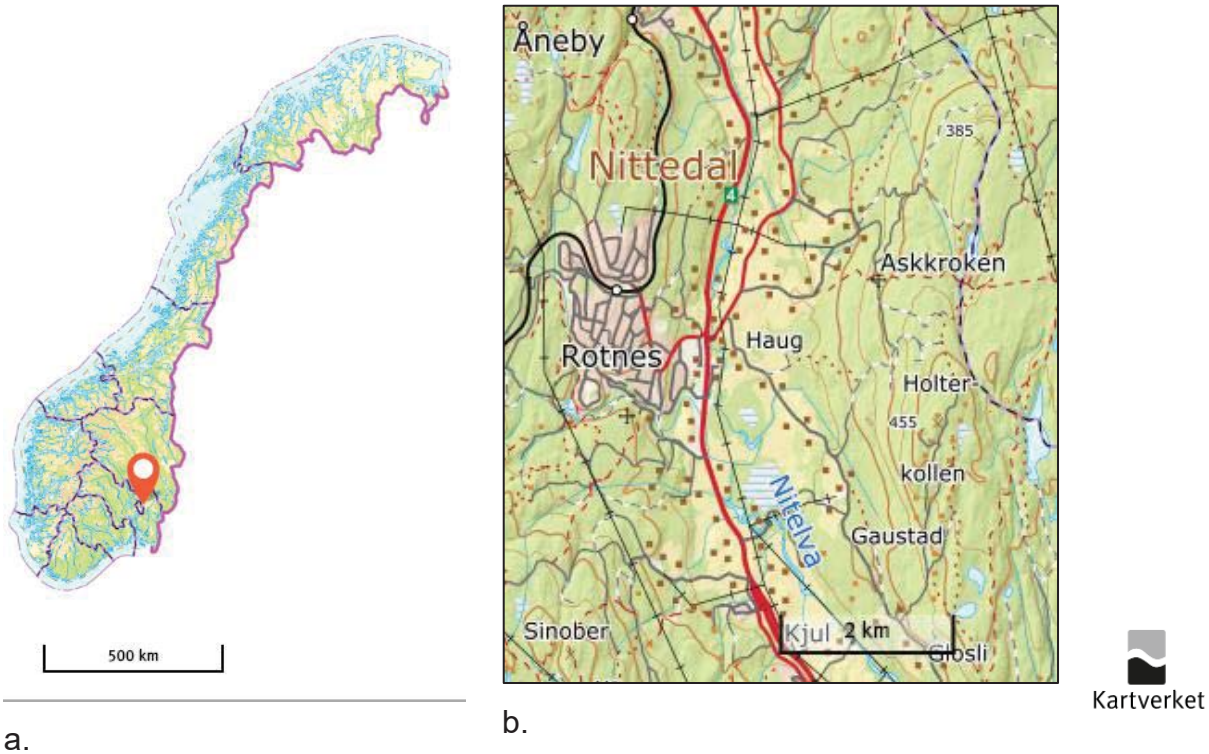
Adult female *M. brandtii* and *M. mystacinus* from neighboring colonies were captured using mist nets and radio tagged for VHF radio-telemetry surveys. The shortened night periods and light northern nights, in addition to the legal ability move through the landscape with limited restriction from crossing privately owned land in Norway (Friluftsloven 1957), made it possible for our research team to physically follow bats as they foraged to collect precise foraging locations. Roost and colony locations were also recorded. Molecular genetic analysis of feces from captured bats was used to verify in hand identification and to explore the diet of the *M. brandtii* and *M. mystacinus*. I used a resource selection function analysis to relate foraging habitat use of *M. brandtii* and *M. mystacinus* to the 3-dimensional vegetation structure of habitat from aerial Light Detection and Range (LiDAR) remote sensing data.

2 Material and Methods

2.1 Study Area

The study took place between June and August of 2018 in the municipality of Nittedal. Nittedal is located in southeastern Norway (Figure1). This area is made up of a mosaic of landscape types that includes agricultural fields, spruce forest, mixed deciduous forest, cultural landscapes and rocky terrain as well as riparian forest areas. The majority of the field work was done in a valley east of the river Nitelva that included the large hill Holterkollen (peak elevation of 455 m; Figure1).

This area was selected in part because it is conveniently located near the Norwegian University of Lice Sciences and because it is an area where previous bat research has been done (Siljedal 2018, Kristiansen 2018). Furthermore, the varied landscape and topography in this area provided the opportunity to study bats in different habitat types.



a. b.

Figure 1. Maps of the study area within Norway (a) and of the study area (b). Images downloaded from https://www.norgeskart.no/.

2.2. Study Species

M. brandtii and *M. mystacinus* (Figure2) are common European species that have overlapping ranges across the continent and are categorized as “Least Concern” by the IUCN (Hutson et al. 2008, Coroiu 2016). Both species maintain the same status under the Norwegian National List for Species (Wiig et al. 2015, Størkersen et al. 2018). Both species are distributed throughout the country, with their overlap mostly being in the southeastern Norway, and their northernmost records reaching Trøndelag county (https://www.Artskart.artsdatabanken.no).

Figure 2. *Myotis brandtii* (left) and *M. mystacinus* (right). Photographs by Jeroen van der Kooij.

Previous to 1970, *M. brandtii* and *M. mystacinus* were not distinguished as separate species. Genetic research in addition to a thorough study of morphological features revealed that though the two species appear very similar, they are separate (Gauckler and Kraus 1970, Hanák 1970, Baagøe 1973). Further research revealed that the Whiskered bat, *M. mystacinus* as it was described previous to 1970, actually consists of four *Myotis* species found throughout Europe that share very similar morphological features (Benda and Tsytsulina 2000, von Helversen et al. 2001).

Given their distant genetic relatedness, the morphological similarities between these two species are all the more striking. Several studies comparing in-hand identification of *M. brandtii* and *M. mystacinus* to molecular genetic analysis of individuals have found a combination of physical characteristics can be used for reliable in-hand identification that require recognizing variation in diagnostic features such as penis shape, dentition, forearm length, tragus shape, and pelage coloration (Berge 2007,

Lucan et al. 2011). However, incorrect field identification was made in both studies and it is important to note that even with experts it can be difficult to distinguish between *M. brandtii* and *M. mystacinus* in the hand.

The ecological roles and life history strategies of *M. brandtii* and *M. mystacinus* exhibit many similarities as well. Both species give birth to a single pup (occasionally two pups) and raise them in maternity colonies that typically include 20-60 individuals. The mothers give birth in June and the colonies have typically dispersed by August, with some variation throughout their ranges (Dietz and Kiefer 2016). Maternity roosts of these species are frequently located in anthropogenic structures such as barns, houses, and outbuildings and as well as bat boxes and occasionally in trees (Berge 2007, Buckley et al. 2013, Dietz and Kiefer 2016).

Myotis species are notoriously long lived, often found surviving into their 30s or even longer in the wild (Gaisler et al. 2003, Foley et al. 2018, White et al. 2019). The oldest individual bat ever recorded was a male *M. brandtii* which was found in a Siberian hibernaculum at the age of 41 years old (Podlutsky et al. 2005).

During the winter, these species are typically found hibernating in caves and mines throughout their range (Buckley et al. 2013, Belkin et al. 2015, Dietz and Kiefer 2016). Both species are frequently seen during annual hibernacula counts conducted throughout the Oslo fjord area (Kristiansen 2018). However, the number of bats found during the annual surveys make up a small proportion of those that are detected on the landscape during the summer (Jeroen van der Kooij, personal communication). It is unclear rather or not *M. brandtii* and *M. mystacinus* in Norway are using alternative hibernating sites that are more difficult to monitor or if they are migrating away from their summer territory and using foreign hibernacula sites. One study in Norway found *M. mystacinus* roosting in both screes and rock crevices during the winter, presumably as hibernacula sites (Michaelsen et al. 2013). The longest recorded movements for *M. brandtii* and *M. mystacinus* are 618 and 625 km respectively but these longer movements are rarely observed, and it is currently thought that both species are mostly residential species with some partial migration (Hutterer et al. 2005).

The wing shape and echolocation characteristics of *M. brandtii* and *M. mystacinus* are nearly identical. The wing morphology of these species is described as having a moderate wingspan with low aspect ratio and there is no significant difference found between the species (Norberg and Rayner 1987, Berge 2007). Baagøe (1987) studied the wing morphology of Scandinavian bat species and also found that *M. brandtii* and *M. mystacinus* shared nearly identical wing shape which they suggested predisposed them to slower flight and moderate maneuverability. Both species use frequency modulated foraging echolocations with similar ranges while foraging; distinguishing between the two species using only acoustic recordings is unreliable (Vaughan et al. 1997b, Barataud 2015). Thus, in hand identification and/or some form of molecular genetic analysis of these species is required to have any level of confidence in distinguishing between them where their ranges overlap. Genetic analysis of feces from colonies is sometimes used to determine species roost occupation for this reason as well (Boston et a. 2010). In combination, wing morphology and echolocation of *M. brandtii* and *M. mystacinus* suggest that they forage in forested areas and forest edge with the ability to move through cluttered areas with relative ease (Norberg et al. 1987, Aldridge and Brigham 1988, Berge 2007).

2.3 Bat Captures

2.3.1 Capture Sites and Bat Processing

All bat trapping was done under the supervision of Jeroen van der Kooij, Clare Stawski, Rune Sørås, and/or myself who have a combined experience of over 40 years working with bats, through permits provided by the Norwegian Environmental Agency. All crew members were up to date with rabies vaccinations and trained to handle bats appropriately with minimal stress to the animal during collection, processing and release.

Trapping sites were selected in flight corridors where it would be possible to funnel the bats toward nets and traps such as over water bodies or along forest paths (Kunz and Parsons 2009). Trapping also took place at maternity colonies when it was necessary to follow the movements of bats specifically from these colonies or during periods when it was challenging to capture target species on the landscape.

The trapping efforts began at sunset and continued until bat activity dropped or up to 5 hours after sunset. During the survey period, the nets and/or traps would be checked at least every 10 minutes (MacCarthy et al. 2006). Handheld heterodyne bat detectors, hereafter referred to as bat detectors (Magenta Bat 5 Heterodyne Bat Detector and Peterson Ultrasound Detector), were used at trapping sites to monitor bat activity and to notify the field crew of when bats were captured in the traps. Bats were identified to species, evaluated for reproductive status, sexed, aged and forearm length as well as weight were measured. Manual as well as electronic calipers were used to take right forearm measurements from the wrist to the elbow and recorded out to the first decimal place. Weight was recorded using a kitchen scale that measured out to one decimal place. Bats were stored in a tared paper tube during weighing. When possible, the feces of captured bats were also collected and stored in ethanol for further molecular analysis.

Captured bats were kept in cloth bags while awaiting processing. The bats were released as quickly as possible in the vicinity of where they were captured. In situations when the bats were held longer due to busy trapping nights or because they were tagged, bats were kept warm by being carried on the person of field crew members in order to reduce the chances of bats going into torpor. Bats were often provided mealworms and water in order to make up for lost foraging time.

Trapping efforts were centralized around monitoring two maternity colonies that had been located in the previous summer by another research project (Siljedal 2018). Care was taken to not expose the colonies to extreme stress, especially during more critical periods in the maternity season such as the period at the end of pregnancy and the start of lactation. Netting efforts largely took place on the landscape rather than directly at the colony in order to further reduce stress.

Each bat that was captured had high resolution photos taken of both wings in order to identify individuals from unique patters of collagen-elastin bundle as well as scarring or other pathologies (Figure3). These photos can be inspected using the human eye to recognize unique patterns with a high success rate (Amelon et al. 2017). This made it possible to collect valuable information about individual bats (movements between

roosts and mist net sites, aging, injuries, etc.) while limiting the chance of injury from marking methods such as banding.

Adult female of target species, *M. mystacinus* and *M. brandti,* were selected for radio-tagging. Reproductive individuals and those of higher weights were prioritized for tagging. The transmitter was attached to the back of the bat just below the interscapular area using skin binding glue (Medical latex glue, Sauer Hautkleber, Manfred Sauer, Germany). Tag weight and approximate glue weight in relation to the weight, physical condition and reproductive health of the bat were taken into consideration before affixing a transmitter to any bat. The total weight of the tag (including weight of glue) did not exceed 7% of the bat's bodyweight (Aldridge et al. 1988, Neubaum et al. 2005).

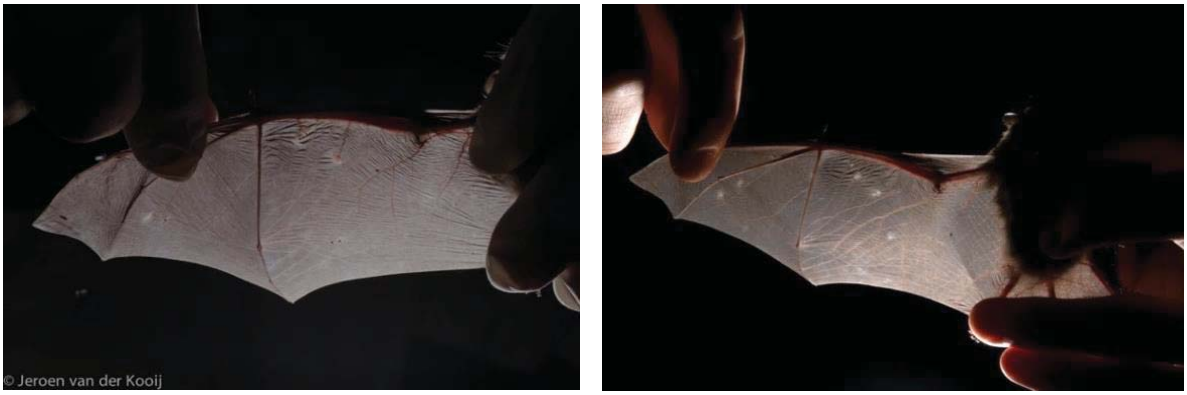


Figure 3. The right wing of a female *M. mystacinus* (Steffi) that was captured on 13.06.2018 (left photo), and then later recaptured and radio tagged at different capture site on 04.08.2018 (right photo). Photographs taken by Jeroen van der Kooij and Rune Sørås.

2.3.2 Distinguishing between *M. brandtii* and *M. mystacinus*

Several morphological features need to be taken into consideration in order to ensure a relative level of certainty in distinguishing between *M. brandtii* and *M. mystacinus* in the hand (Berge 2007, Lucan et al. 2011; Table 1). The primary characteristics used in this study were dentition and penis shape, while forearm length, pelage and skin coloration, and tragus shape were also taken into consideration, especially when evaluating females or sub-adults.

One of the strongest features used to determine between the two species is dentition (Baagøe 1973, Berge 2007, Figure 4). This feature has even been observed in skulls bats of these species that date back to the Holocene (Rybář 1976). Teeth can be used regardless of sex of the animal and is more consistent and easier to observe than other morphological features such as pelage color, facial characteristics or tragus shape which are more likely to vary amongst individuals and more subject to observer error.

The dentition of *M. brandtii* is distinguished from *M. mystacinus* by a pronounced protocone that extends off of P_4 in the maxilla towards P_3 and P_2. Also, in *M. brandtii* P_3 and P_2 in the maxilla and P_2 and P_3 in the mandible are more similar in size, while in *M. mystacinus* P_3 and P_3 tend to be smaller in relation to P_2 and P_2. A loupe was used to better visualize the teeth of bats.

Penis is the other primary feature used as a diagnostic tool (Figure 5). *M. brandtii* has a club shaped penis whereas *M. mystacinus* have a longer, thinner member. However, sub-adult *M. brandtii* may have not acquired the club shape yet and could appear more like *M. mystacinus.*

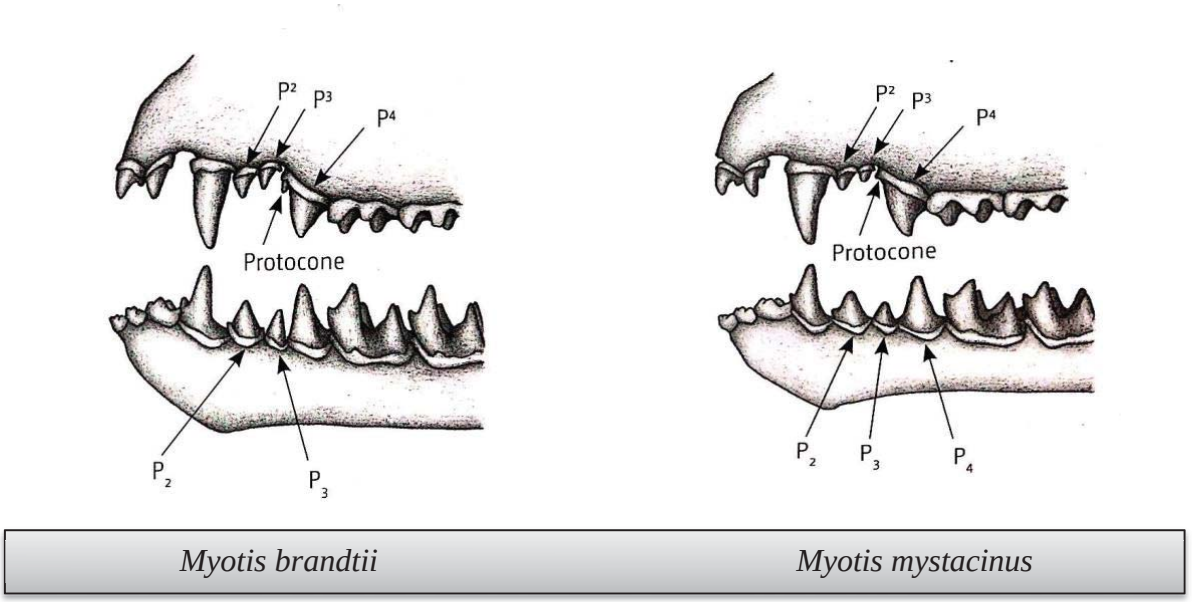


Figure 4. Diagram of the dentition of the maxilla of two *Myotis brandtii* and *M. mystacinus* from Dietz and Kiefer (2016). “P” refers to a given premolar.

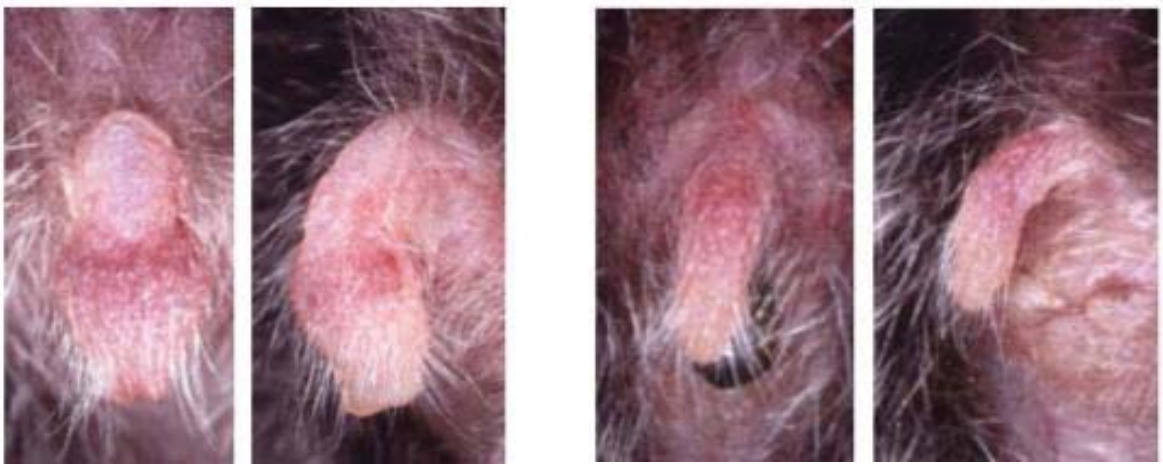


Figure 5. Penis shape in *Myotis brandtii* (left) and *M. mystacinus* (right). Images from Dietz and Kiefer (2016).

Table 1. A review of secondary morphological features of the bat species *Myotis brandtii* and *M. mystacinus*.

Diagnostic Feature	*M. brandtii*	*M. mystacinus*	Notes
Forearm length range (mm)	33.4 – 36.0[1] 33.8 – 38.2[2] 34.7 – 36.9[3]	32.9 – 35.1[1] 32 – 36.5[2] 33.5 – 35.9[3]	*M. brandtii* is slightly larger than *M. mystacinus* but there is often overlap between the two species.
Tragus shape	Convex posterior edge	Concave or straight posterior edge	Difficult to observe but with a high degree of effectiveness for distinguishing between the two species[1].
Tibia Length (mm)	15.1 – 17.3[3]	15.3 – 16.5[3]	Rarely included in standard measurements.
Thumb claw length range (mm)	1.5-2.3[1]	1.2 – 2.1[1]	Difficult to measure but with a high degree of effectiveness for distinguishing between the two species[1].
Pelage color	Adults: golden-brown pelage Subadults: dark brown pelage.	Adults: dark brown dorsal hair and a dark face. Sub adults: dark brown pelage	There is often much variation in pelage color and perception of color can vary between observers or lighting.[1, 2]

[1]Berge 2007, [2]Dietz and Kiefer 2016, [3]Lucan et al. 2011

2.4 Molecular Fecal Analysis

2.4.1 Overview

Feces were collected at mist net sites, from cloth bags where individual bats were temporarily held while awaiting processing. The feces were removed from the bat bags while processing the bat and placed in 2 ml tubes of 100% ethanol where the feces were stored for a minimum of 24 hours and up to a year. Each sample is associated with an individual bat capture. The feces were then transferred on to dry, odorless wipes and stored in individual 50 ml falcon tubes that were filled to approximately ⅓ with silica beads. Feces used in the following analyses were collected throughout the 2018 field seasons as well as from previous research done with the same population in the summer 2017. The samples included feces from *M. brandtii* and *M. mystacinus*, as well as from *M. daubentonii, E. nilssonii* and *P. auritus* (Table 2). The majority of samples came from the month of June for both 2017 and 2018. Only *M. brandtii* and *M. mystacinus* were used for bat species identification, while all fecal samples were included in the dietary analysis.

The genetic analysis took place in the fall of 2019 at the Konrad-Lorenz Institute Ethology in Vienna, Austria through a collaboration with the Ecological Genetics Working Group under the supervision of Dr. Steven Smith and his research team.

Table 2. Summary of the number of fecal samples for each species that were included in this study. *One sample was collected in 2019. Target species presented first.

Species	English name	2017	2018	Total
Myotis brandtii	Brandts' Bat	4	14	18
Myotis mystacinus	Whiskered Bat	13	21	34
Eptesicus nilssonii	Northern Bat	1	2	3
Myotis daubentonii	Daubentons' Bat	9	1*	10
Plecotus auritus	Brown Long-eared Bat	4	4	8
All Species		31	52	73

2.4.2 DNA Extraction

DNA was extracted from all fecal samples using QIAamp® PowerFecal® DNA Kit (Catalog number 12830-50, QIAGEN, Hilden, Germany) following the instruction provided in the manual (version August 2016). I made several modifications to the manual for my purposes in the following steps:

1.) Approximately 1-2 pellets (10-50 mg) were used as starting material.
5.) Instead of a Vortex Adapter tube holder, I used a TissueLyser LT by QIAGEN at 50 oscillations per second for 10 minutes.
17.) The sample was centrifuged for 2 minutes at 13000 x g.
19.) 100 μl AE buffer was used and allowed to stand in the spin column for 10 minutes before the final centrifuge step.

Extraction negative controls were included in each round of extraction in order to detect contamination.

2.4.3 Bat species determination and Insect meta-barcoding

For amplifying bat DNA in a polymerase chain reaction (PCR) I used the SFF primer pair (SFF-145f: 5′- GTHACHGCYCAYGCHTTYGTAATAAT-3′ and SFF-351r: 5′- CTCCWGCRTGDGCWAGRTTTCC-3) described by Walker et al. (2016). PCRs contained 5 μl of undiluted DNA in a 20 μl reaction with a final concentration of 2.5 μl 10x Buffer (Solis BioDyne), 2.5 μl, magnesium chloride [25 mM], 2.5 μl dNTP's [2 mM], 0.5 μl of each primer [10 μM], 0.3 μl BSA[50 mg/ml], 11 μl PCR water, 0.2 μl firepol [5 U/μl] (Solis BioDyne). The thermal conditions of this PCR are as follows: 95 °C for 5 minutes, follow by 35 cycles - 95 °C for 30 seconds, 58 °C for 30 seconds, 72 °C for 30 seconds-, then 72 °C for 5 minutes, and 12 °C indefinitely. PCR products were store at 4 °C.

All extraction negative samples and a non-template control sample were included in each PCR run to control for contamination free DNA extraction and PCR amplification. All PCR products were checked for amplification on a 1% agarose gel including

GelRed (Biotium, Inc.; Hayward, California) following the manufactures instructions. All samples that appeared positive on the gel (neither of the control samples did) were sequenced on an ABI 3130xl Genetic Analyzer (Applied Biosystems, Foster City, California) using BigDye Terminator V3.1 (Applied Biosystems, Foster City, California) following the manufacturer's instructions. Forward as well as reverse sequences were run for each sample. A second sequencing reaction was performed to verify the results.

To amplify arthropod DNA for the dietary analysis, I used the primer set ZBJ-ArtF1c and ZBJ-ArtR2c, developed by Zeale et al. (2011) with overhang at the 5' end (indicated by asterisks) for subsequent indexing PCR (see below).
ZBJ-ArtF1c: 5′-*TCG TCG GCA GCG TCA GAT GTG TAT AAG AGA CAG* AGA TAT TGG AAC WTT ATA TTT TAT TTT TGG-3′.
ZBJ-ArtR2c: 5′-*GTC TCG TGG GCT CGG AGA TGT GTA TAA GAG ACA G*WA CTA ATC AAT TWC CAA ATC CTC C-3′.

Each PCR was set up in a 20 μl reaction as followed: 12 μl of undiluted DNA, 5 μl AllTaq Mastermix (Qiagen, Hilden, Germany), 0.5 μl of each primer [10 μM], 0.3 μl BSA [50 mg/ml], 1.7 μl PCR water. The thermal conditions of the touchdown PCR are as follows: 95 °C for 5 minutes, followed by 16 cycles - 94 °C for 5 seconds, 61 °C for 15 seconds, 72 °C for 10 seconds - with a 0.5 °C decrease with each cycle, followed by 24 cycles - 94 °C for 5 seconds, 53 °C for 15 seconds, 72 °C for 10 seconds. PCR products were stored at 4 °C.

To pool all samples in one NGS sequencing run, arthropod PCR products were individually labeled in an indexing PCR using the i7 and i5 primer system. The plate setup is shown in Table A5 and the primer sequences in table A6 (Appendix A2). Each indexing PCR was set up in a 20 μl reaction as followed: 1 μl of arthropod PCR product, 5 μl AllTaq Mastermix (Qiagen, Hilden, Germany), 0.5 μl of each primer [10 μM] and 13 μl PCR water. The PCR temperature conditions are as follows: 95 °C for 2 minutes, followed by 15 cycles - 95 °C for 5 seconds, 55 degrees for 15 seconds, 72 degrees for 10 seconds. Indexing PCR product was stored at 4 °C.

Extraction negative samples and non-template control samples were included in all PCR runs and showed no sign of amplification on agarose gels.

To pool all indexed PCRs equimolarly, the SequalPrep™ Normalization Plate (Invitrogen, Carlsbad, California) was used, following the instructions provided in the manual. The advantage of using this normalization plate is the accurate normalization of the PCR product to 1.5 ng/μl and a parallel clean-up of the PCR product. All cleaned products were pooled in one single tube and sent to the Vienna BioCenter for a 150 paired end sequencing run on an Illumina NextSeq550 platform.

2.5 Tracking Effort

VHF telemetry tags used in this study were Biotrack PIP4 tags that weighed 0.31 or 0.32 g with frequencies in the range 142.000-142.330 MHz. Sika receivers (Biotrack, UK) were used with handheld flexible 3 element and 5 element YAGI antennae (Biotrack, UK). In total 10 *M. brandtii* and 12 *M. mystacinus* adult females were radio-tagged and tracked on average 7.4 and 6 days respectively.

Once the tagged bat was released, an individual researcher or a team would follow the bat on foot or by car. Each bat would be tracked for the entire foraging period every night until the life of the transmitter ended (approximately a week) or until it was necessary to prioritize tracking another individual. Day roost sites were located during the day or at the very end of the night when the bat had stopped foraging and returned to a day roost. When bats were observed roosting at alternative sites for prolonged periods during the night, these sites were recorded as night roosts.

A combination of techniques were utilized to study the foraging behavior of tagged bats. Whenever possible, a single person would ‘home in’ on an individual bat (White and Garrott 2012). One or more researchers would directly pursue a tagged bat in order to get as close as possible to the bat. When the researcher was close enough to see the targeted bat or pick up its echolocation on the bat detector, these were considered ‘onsite plots’. The frequency and call characteristics as interpreted through the bat detector were used to verify that the bat seen foraging was indeed a *Myotis* species.

When it was not possible to stay in close enough proximity to collect onsite plots, other techniques were used such as triangulation, cross bearings and a telemetry tower. Triangulation is the process by which three separate bearings on the same target are taken from three different locations at approximately the same time and the resulting triangle created by the intersection of these bearings are used to estimate the position of the target. Cross bearings are acquired by taking two bearings on a single target from two different positions at approximately the same time. The intersection of the two bearings can be used to estimate the position of the target. In situations when a bat was entirely out of range to researchers with handheld antennae, a telemetry tower would be used to give tracking crews more information about the bat's position. The telemetry tower consisted of a 5 element YAGI antenna that was attached to a 6 m tall pole that could be erected, preferably at higher elevations, in order to pick up signal from transmitters as far as 2 kilometers away. High elevation points were also utilized by crew members without the tower to increase the range of their antennae.

Each observation of a tagged bat being tracked included the GPS location of the observer, the date and time of the observation, the strength of the transmitter signal being received, the gain and noise that was set on the VHF receiver as well as the azimuth of the observation. Additionally, any time that a tagged bat was visually observed or was aurally observed using bat detectors, this was also recorded.

Through previous research conducted with project SCANDBAT (Siljedal 2018), the VHF equipment was tested to determine the range of the instruments in the study area. Through these tests, we have determined that any observation made of a bat when the signal being received was at least 45 in strength, would place the bat within approximately 25 m of the observer. Any observation gathered with 45 signal strength or lower, I have classified as a "fix." Any observation that included visual or aural verification of the tracked bat, I have classified as an "onsite plot" and places the observer within approximately 10 m of the bat.

2.6 Roost Sites

Roosts of tagged bats were located by tracking bats throughout the night until they stopped foraging or by tracking tagged individuals during the daytime. Each roost was classified as a house, structure, tree or rock crevice. A ‘house’ is defined here as a building where people were actively living or working inside year-round whereas ‘building’ refers to other structures such as sheds, cabins or barns with limited or intermittent human use. The primary colonies located by Siljedal (2018) are referred to here as HM and HB where “H” indicates that the colony was located in a house and “B” and “M” refer to the primary species inhabitants: *M. brandtii* and *M. mystacinus* respectively. For further details relating to bat roost monitoring and exit counts from these populations, refer to Birkeland (2019).

Roosts were monitored with exit count surveys on the first night after a tagged bat was located there, and thereafter throughout the summer in conjunction with research objectives of a separate master thesis (Birkeland 2019). Exit count surveys consisted of one or more observers monitoring a roost site at sunset to determine how many bats were entering and exiting the roost. Observers were equipped with a bat detector in order to improve the chance of noticing when a bat was approaching or leaving the roost as well as radio telemetry equipment if there was a tagged bat inside of the roost. Any site with three or more bats exiting was considered a potential maternity colony and would be monitored for subsequent exit count surveys. If a tagged bat did not exit a roost for several nights in a row, this tag was designated as no longer active and assumed to be shed in the roost. Whenever possible, efforts were made to retrieve tags from roost sites or other shedding locations.

2.7 Data Analysis

All data analysis was done using R Studio Version 1.2.5033 (R Core Team 2019).

2.7.1 Body Size Metrics

The right forearm length is one of the few diagnostic features used for distinguishing between *M. brandtii* and *M. mystacinus* that can be measured with relative ease. Analysis of variance (ANOVA) was used to test if right forearm length differed between the two species, and to determine if there was difference in size between male and female bats. Due to the fact that sub-adults make up a very small portion of the sample, age was not included in analyses and instead the focus is the variation of forearm length between species and between males and females within species. Weight was not included as a factor due to the variability of weight throughout the night while foraging as well as in relation to reproductive status of female bats throughout the maternity season.

2.7.1 Foraging Habitat Selection and Home Range Size

To determine if the probability of use of a foraging site was influenced by habitat variables defined from aerial LiDAR (Light Detection and Ranging), and if habitat use differed between *M. brandtii* and *M. mystacinus*, I used the method of Resource Selection Function (RSF) analysis *(*Manly 1985, Manly et al. 2002). I chose to include all fixes (foraging locations) where the observer was within 25 m of the tagged bat in the RSF analyses.

Habitat resources were quantified in the form of LiDAR data. LiDAR is an active remote sensing technique that uses a sensor to emit and simultaneously measure brief laser pulses in quick succession to illuminate an object and subsequently model a 3-dimensional representation of that space (Vierling et al. 2008, Figure 6). LiDAR systems can be used in a variety of contexts to measure terrestrial as well as aquatic spaces and is an increasingly popular method used in ecological studies for relating wildlife to their environment (Davies and Asner 2014, Ciuti et al. 2018). In the case of this study, the data used was collected using airborne LiDAR systems which mapped the vegetation of the study area in the course of a forestry inventory project (Terratec

2015). This tool has been found to be especially useful for understanding the relationships between avian wildlife and their continuous 3-dimensional habitat which inherently have a strong ecological link to vegetation structure such as forest canopy which are challenging or improbable to measure adequately on a large scale using categorical, 2-dimensional landscape remote sensing techniques such as satellite imagery (Davies and Asner 2014, Eldegard et al. 2014).

The discrete return LiDAR data was collected by Terratec AS between November 11 and December 3, 2015 using Lecia 1200 GPS receivers to perform RTK-GNSS (Real Time Kinematic – Global Navigation Satellite Sensing) with the measurement method CPOS (Centimeter position level of accuracy). Further details regarding LiDAR data collection can be found in the report from Terratec (2015). Analyses included habitat data with the resolution of 16 and 250 m_2 in raster stacks which covered the study area.

RSFs are structured by defining resources as either used/unused or used/available as a means of expressing the selectivity of positions (Boyce & McDonald 1999, Boyce et al. 2002, Manly et al. 2002, Nielsen et al. 2002, Nielsen et al. 2005). Due to the nature of the VHF telemetry data collected in this study, the models were built on defining resources as either used or available since it would be impossible to know if areas were specifically unused. Used areas were defined by the actual observations collected in the field while available areas were defined by creating random points from within 95% minimum convex polygons (MCPs) for each individual bat (Worton 1987, see Figure 7). For each individual bat, the number of random points was equal to the number of real observations.

All the LiDAR variables presented in Table 3 were included in full (most complex) RSF models. The full datasets used during these analyses (including used and available observations) for the 16 m_2 resolution and 250 m_2 resolution contained 2970 and 2988 observations respectively. Variation is due to removal of missing values for one or more of the LiDAR variables.

Many of the LiDAR variables were strongly correlated (Appendix1, Tables A2 and A3). To deal with collinearity among LiDAR variables, I first fitted a generalized linear mixed

model (GLMM) for each species, with a binary response variable (Y = 1 = used habitat and Y = 0 = available habitat) and all the LiDAR variables in Table 3 as fixed-effects predictors. Bat identity was included as random effect to account for repeated observations of the same individuals. I then calculated variance inflation factors (VIF) for each variable. I created a function to sequentially drop the variable with the largest VIF until all variables had VIF > 2, as recommended by Zuur et al. (2010). Following this procedure, I found that I could include the candidate LiDAR variables zmax, zq10, zq20, zq30, zq40 and zcv in the full (most complex) model for both *M. brandtii* and *M. mystacinus.*

To analyze if habitat use differed between *M. brandtii* and *M. mystacinus* and was influenced by habitat structure, as quantified by LiDAR variables, I fitted a GLMM with a binary response variable (Y = 1 = used habitat and Y = 0 = available habitat) and species, zmax, zcv, zq10, zq20, zq30, zq40, and the interactions species×zmax, species×zcv, species×zq10, species×zq20, species×zq30, species×zq40 as fixed-effects predictors. Bat identity nested within species was included as random effects to account for repeated observations of the same individuals. After fitting the full (most complex) model, I carried out model selection based on Akaike's information criterion (AIC) (Akaike 1974, see e.g. Bolker et al. 2008), using the dredge function in the R package MuMIn (Bartón 2020). The AIC encourages parsimony by adding a term to penalize more complex (larger number of parameters) models (e.g. Bolker et al. 2008). According to Burnham and Anderson (2002), models with delta AICc values < 4 should be considered competing models to the model with the highest AIC weight.

As an alternative to model selection based on AIC, I also used a hypothesis testing approach for model building and model selection. Though both methods are frequently used for RSF modeling, the AIC approach is often assumed to be better suited to radio-telemetry data with many covariates (Boyce et al. 2002) and so is the method that I chose for my primary results, while the methods and results from the hypothesis test approach are listed in Appendix A1.

Home ranges were defined by 95% minimum convex polygons (MCPs) made up of fixes, i.e. observations within 25 m of a tagged bat. To test if there was a difference between *M. brandtii* and *M. mystacinus* in home range size, I fitted a linear model with

individual MCP area as response and species, the number of fixes and their interaction as explanatory variables.

Table 3. List of LiDAR (light detection and ranging) variables included in the resource selection function analysis (RSF) and their descriptions relative to vegetation structure. The LiDAR variables were standardized before fitting the RSF models; each variable was 'scaled' by subtracting the mean and dividing by the standard deviation: (x - mean(x)) / sd(x).

LiDAR Variable	Description
zmax	Maximum height
zmean	Mean height
zsd	Standard deviation of height distribution
zcv	Variance (zsd/zmean)
pzabovezmean	Percentage of returns above mean
pzabove(0.5)	Percentage of returns above 0.5.
zq(x)	x percentile (quantile) of height distribution x = 10, 20, 30, 40, 50, 60, 70, 80, 90.

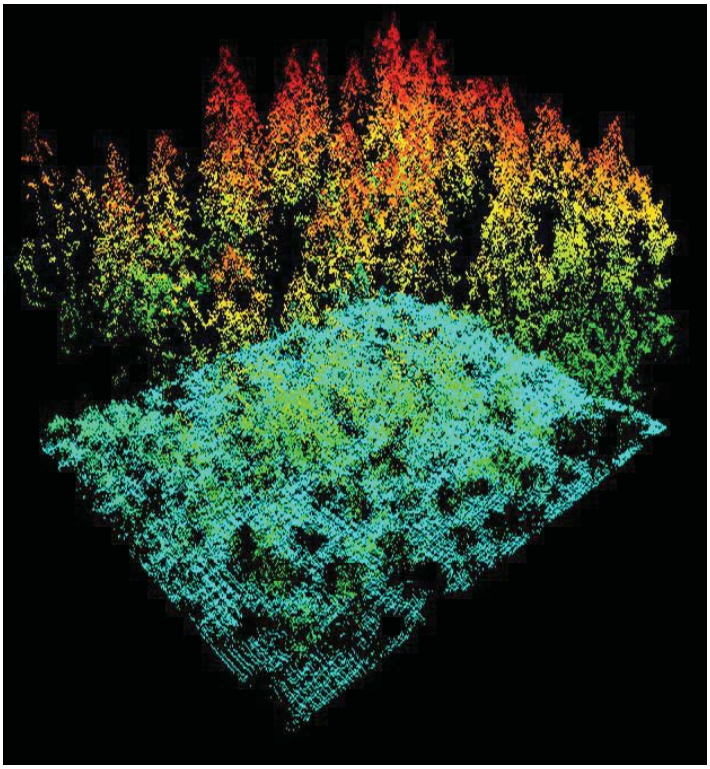


Figure 6. Example of a 3D model of forest created from airborne (sensor mounted on an airplane) LiDAR data. Image provided by the forest inventory and monitoring research group at the Faculty of Environmental Sciences and Natural Resource Management (MINA), Norwegian University of Life Sciences (NMBU) https://www.forestinventory.no/

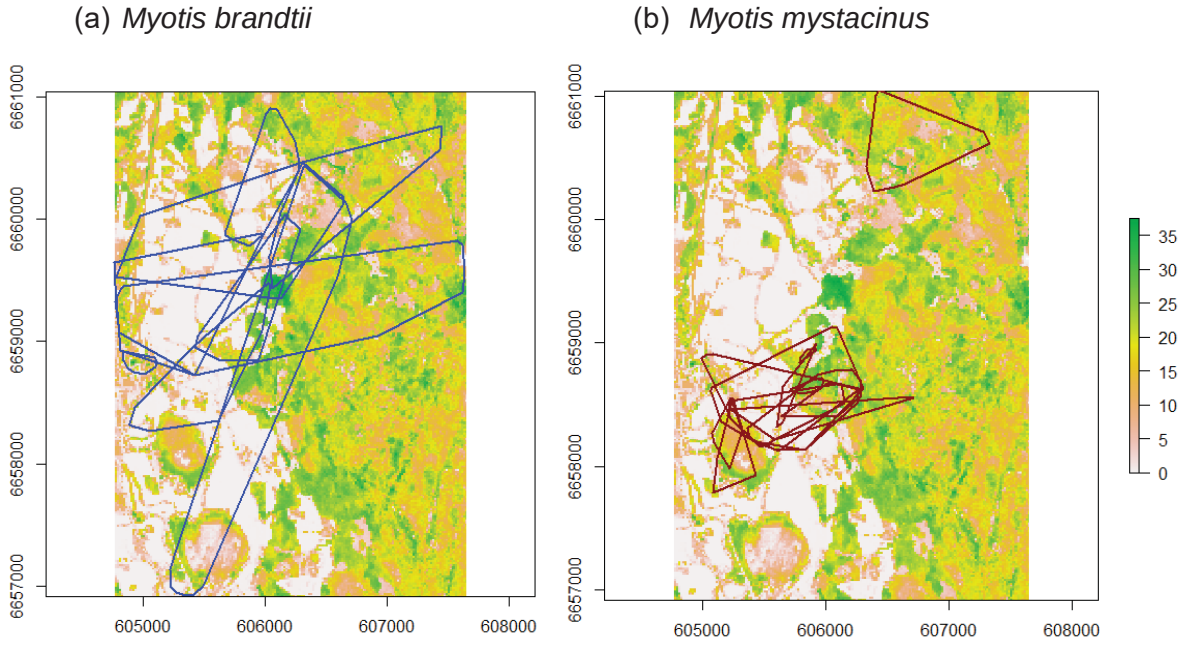


Figure 7. Maps of the 95% minimum convex polygons (MCPs) of individual bats for *Myotis brandtii (*a) and *M. mystacinus* overlaid on a 250 m_2 resolution raster layer of the LiDAR variable zmax (maximum height) within the study area. As a measure of available habitat, I used LiDAR variables in random points generated from within the 95% MCPs of each individual.

3 Results

3.1 Bat Captures

In total there were 221 bats of 6 different species captured from 17 different mist net site locations throughout the study period (Table 4). *M. brandtii* and *M. mystacinus,* or 'target species', made up 76% of the captures. It is important to note that 78% of the captures of target species were female and 93.5% were adults. Capturing of tagged bats was staggered throughout the maternity season period and typically two bats were tracked at once (Table 5).

Table 4. Summary of all bat captures between 05.06.2018 and 28.08.2018. All species are designated as Least Concern (LC) on the Norwegian National List for Species (Wiig et al. 2015). These numbers include recaptures of the same individuals caught on different nights. Target species are presented first.

Species	English name	Norwegian name	Captures
Myotis brandtii	Brandts' Bat	Skogflaggermus	58
Myotis mystacinus	Whiskered Bat	Skjeggflaggermus	110
Eptesicus nilssonii	Northern Bat	Nordflaggermus	9
Myotis daubentonii	Daubentons' Bat	Vannflaggermus	3
Pipistrellus pygmaeus	Soprano Pipistrelle	Dvergflaggermus	1
Plecotus auritus	Brown Long-eared Bat	Brunlangøre	39

Table 5. Summary of the 10 capture sites where tagged bats were captured. Bat individuals with "*" indicate a recapture during the 2018 field season. Individuals that were recaptured in a separate research objective in 2019 are indicated by "**".

Site ID	Captured	Site Description	Bat ID	
			M. brandtii	***M. mystacinus***
N1	05.06	Riparian forest edge		M1, M2
N2	07.06	Pond in cropland	B1	
HM	11.06	House – *M. mystacinus* colony		M2*,M3, M4**
	25.06			M5, M6
HB	13.06	House – *M. brandtii* colony	B2, B3, B9*	
N3	03.07	Coniferous forest edge	B4, B5	
N4	11.07	Mixed forest edge		M6*, M7**, M8,
H2	16.07	*M. mystacinus* colony		M5*, M9**
	20.08			M8* , M11, M12
N5	20.07	Coniferous forest, logging road	B6	
N6	21.07	Coniferous forest, logging road	B4*	M10
	22.07		B7	
N7	04.08	Pond in coniferous forest	B8, B9	
	14.08		B10**	

3.2 Molecular Fecal Analysis

3.2.1 Bat Species Identification

In total, 52 fecal samples from *M. brandtii* and *M. mystacinus*, 19 and 33 respectively, were included in the process of extracting DNA from the feces. Of those samples, 49 successfully amplified in a PCR (Table 6). From the samples that were successfully sequenced in at least one direction, there was one sample with field ID as *M. brandtii* that was revealed to belong to *M. mystacinus.*

Of the 49 samples that were sent through sequencing, there were four that belonged to bats that were radio-tagged. These included bat individuals M2, M1, M6 and M8 (Table 5). The one sample that failed to sequence belonged to a bat, M8, that was recaptured a month after having been tagged and was once again identified in the

hand as *M. mystacinus.* M6, M2 and B10 were also recaptured at other points by SCANDBAT crew members (see Table 5) and had congruent field identification. Of the 21 bats radio-tagged, 80% (n=17) were not included in the molecular genetic analysis of feces.

Table 6. Summary of the results from fecal samples that underwent bat species metabarcoding.

Species	Successful DNA Extraction	Successful Sequencing	Correct Field ID
M. brandtii	17	17	14
M. mystacinus	32	29	29
Total	49	46	43

3.2.2 Diet - Arthropod Species Identification

Due to the COVID-19 pandemic, the final results for arthropod composition in the diet of *M. brandtii* and *M. mystacinus* from the molecular genetic analysis of feces have been delayed in their delivery. These sequences will be analyzed by Dr. Steven Smith's team at the Konrad Lorenz Institute promptly upon their delivery from the BioCenter.

3.3 Tracking Observations

There were 5458 observations in the form of radio telemetry data that were collected from the 22 radio tagged bats with a total of 146 tracking nights for all bats together. 1502 of the observations are classified as fixes, wherein the bat was within 25 m of the observer (Table 7). 682 of the fixes (45%; Table 7) were onsite plots, placing the observer within 10 m of the bat. The remaining observations were made while homing in on bats from farther distances or contributed to bi-angulations, triangulations, tower bearings or other long-range telemetry. For the purposes of exploring the foraging habitat selection and for calculating home range size of these bats, the 1502 fixes were the only observations used in this study and were included in analyses.

Observations made of foraging bats (tagged as well as untagged individuals) which included visual and aural (using bat detectors) observations were inconsistently collected and biased towards individual bats that were more accessible to crew members, so no formal analysis of these observations is included here.

For 40% of the tagged bats, the tracking effort was ended due to a transmitter being shed or otherwise failing. Another 40% were deprioritized when they had been tracked for at least one week. Smith and Racey (2005) recommends tracking foraging bats for at least 5 days in order to obtain adequate habitat data based off research done on *M. natteri.* The U.S. Fish and Wildlife summer survey guidelines for *Myotis sodalis* and *Myotis septentrionalis* (U.S.F.W.S 2020) recommends tracking a bat for at least 7 days or for the life of the transmitter to collect sufficient data on foraging habitat. Thus, our team decided to track bats for at least 5 days and would thereafter consider deprioritizing the individual in order to track other bats. The remaining 20% of tagged bats were no longer tracked due to signal loss, or because the field season ended.

Table 7. Summary of the tagged bats and their tracking history. *M. brandtii* are listed first, followed by *M. mystacinus.* 'Fixes' are observations of tagged bats within 25 m of the observer and 'onsite' plots are fixes, for which the bats were physically seen or picked up on handheld acoustic bat detectors, placing the bat within approximately 10 m of an observer. NR = Non-reproductive, PR = Pregnant, LA = Lactating, PL = Post-lactating.

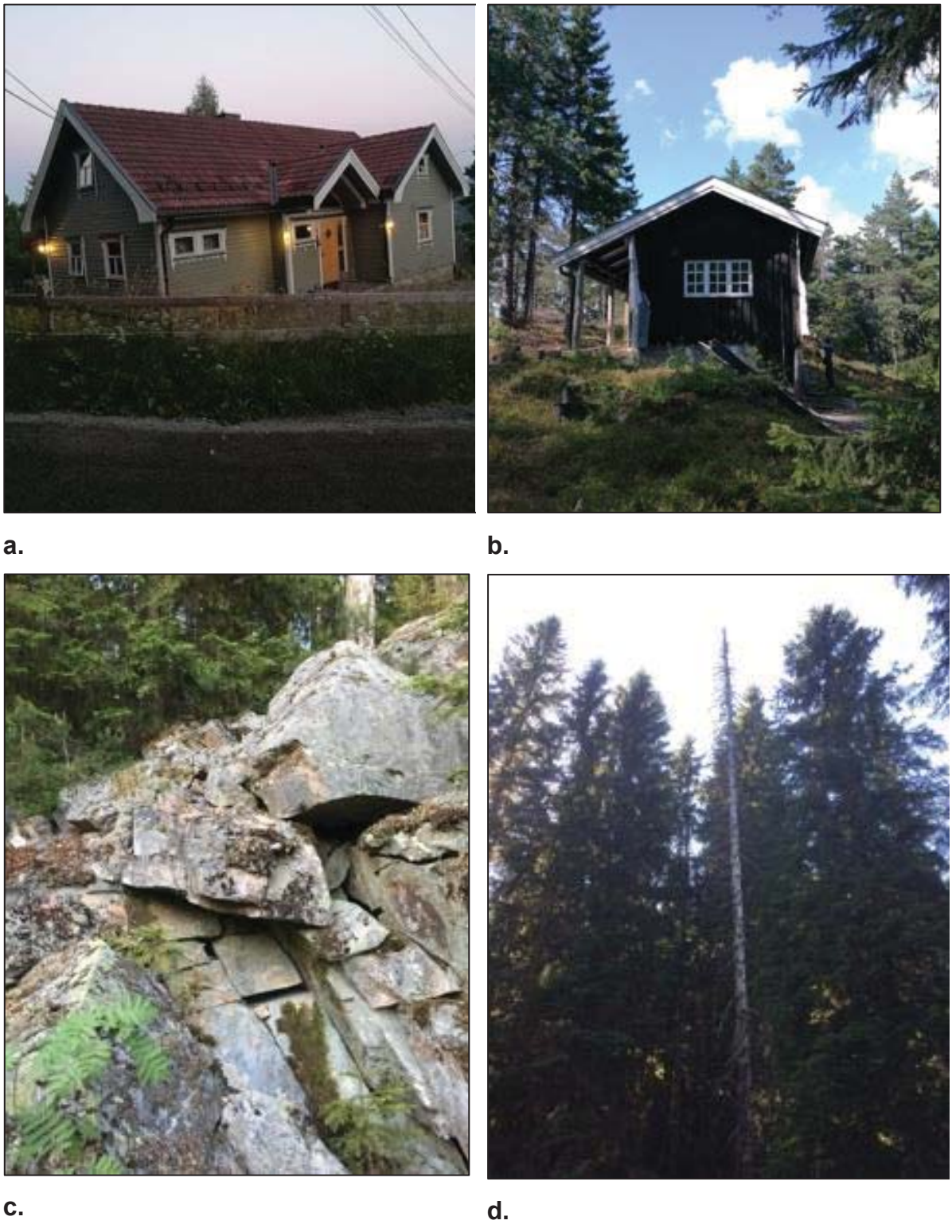
* Indicates bats who were recaptured at other points in the field season with evidence of being reproductive (ex: pregnant, lactating, post lactating). It is difficult to determine if a bat is pregnant in the early stages of the pregnancy and it can be challenging to distinguish post lactating bats from non-reproductive bats in some cases.

Bat ID	Repro. Status	Date Start	Date End	Days	Reason for end	Fixes	Onsite
B1	NR	08.06	10.06	3	Signal lost	0	0
B2	NR	13.06	20.06	7	Transmitter shed	91	39
B3	NR	14.06	26.06	7	Transmitter shed	101	66
B4	LA	05.07	09.07	8	Deprioritized	39	10
B5	LA	05.07	09.07	8	Deprioritized	49	9
B6	NR	20.07	30.07	10	Deprioritized	141	58
B7	PL	23.07	30.07	8	Deprioritized	62	17
B8	PL	04.08	20.08	6	Deprioritized	81	37
B9	PL	04.08	19.08	6	Deprioritized	39	21
B10	NR	14.08	27.08	11	Field work ended	83	72
					M. brandtii total:	**686**	**329**
M1	NR	05.06	11.06	6	Transmitter shed	31	11
M2	NR	05.06	11.06	6	Deprioritized	16	2
M3	NR	11.06	13.06	2	Transmitter failure	8	2
M4	NR	12.06	15.06	4	Transmitter shed	70	39
M5	NR*	25.06	04.07	10	Transmitter shed	134	40
M6	PR	25.06	29.06	5	Transmitter shed	55	17
M7	PL	11.07	15.07	4	Transmitter shed	84	32
M8	NR*	11.07	20.07	9	Deprioritized	117	49
M9	PL	16.07	19.07	3	Transmitter failure	28	5
M10	PL	21.07	30.07	9	Deprioritized	119	32
M11	PL	20.08	27.08	7	Field work ended	64	51
M12	PL	20.08	27.08	7	Field work ended	90	73
					M. mystacinus total:	**816**	**353**
					Total (both species):	**1502**	**683**

3.4 Roost Sites and Social Colonies

Tagged bats were tracked to 30 different roosting sites including houses, structures, trees and rock crevices (Figure 8). The only roosts that were also colony locations were houses which I classify here as buildings where people were living or working throughout the year. Both species were observed using trees at least once as roosts but in these few occasions, the bats roosted alone (Table 8). Two different individual *M. mystacinus* roosted alone in rock crevices on three separate occasions and *M. brandtii* was never observed using a rock crevice as a roost. I found no significant difference in the pattern of roost site use between the two species (Table 8, Fisher Exact test $p = 0.47$). Individual bats were tracked to two roosts on average, regardless of species.

Exit count surveys were performed on all roosts found by tracking tagged bats. Roosts where multiple bats were seen emerging were treated as potential maternity colony sites. The max exit count surveys associated with the colony sites of each species were similar (Table 9). There were 8 different colony sites used by tagged bats that were studied in detail by Birkeland (2019) and continuously monitored throughout the maternity season. The primary colonies, HB and HM, which were located in 2017 through earlier research (Siljedal 2018) had the largest exit counts (Table 9). Other neighboring colonies of similar population size were located for both species (Table 9). Colony sites were located within 2 km of capture sites.



a. **b.**

c. **d.**

Figure 8. Images of the four different types of roost sites that were used by both species. House (a., colony site H7), structure (b.), rock crevice (c.), tree (d.). Photo b. was taken by Rune Sørås. Photos a., c., and d. taken by April McKay.

Table 8. Summary of the roost selection and number of days the bat was observed using each roost type.

Roost Type	*M. brandtii*	*M. mystacinus*
House	8	7
Building	4	4
Rock crevice	0	3
Tree	2	2
Total Roosts:	14	16

Table 9. Summary of the colonies of each species with the maximum number of bats counted exiting that roost for one night. 'H' is used here to indicate 'House'; HB and HM were the primary colonies of *M. brandtii* and *M. mystacinus* respectively. *A tagged *M. mystacinus* also roosted in the *M. brandtii* colony H13.

M. brandtii	Exit Count	*M. mystacinus*	Exit Count
HB	49	HM	35
H7	34	H1	24
H13*	16	H2	23
H6	5	H9	5

3.5. Body Size Metrics

I found a significant difference in body size (right forearm length) between *M. brandtii* and *M. mystacinus* (analysis of variance: $F_{1,165}$= 60.0, p < 0.0001). The average forearm length was 35.19 ± SD 1.14 mm for *M. brandtii* and 33.91 ± 1.04 mm for *M. mystacinus*. When I also included sex and the species × sex interaction as fixed effects explanatory terms in the statistical model, I found that the species differed in the degree of sexual size dimorphism (species: $F_{1,163}$= 60.2, p < 0.0001, sex: $F_{1,163}$= 1.5, p = 0.22, species × sex: $F_{1,163}$= 4.1, p = 0.046). This was confirmed by separate analyses of the two species; there was no significant difference in body size between males and female *M. brandtii* (sex: $F_{1,55}$= 1.3, p = 0.26, Figure 9), whereas male and *M. mystacinus* tended to have shorter forearm lengths than females ($F_{1,108}$= 3.6, p = 0.062, β_{males} = - 0.44 ± 0.23 SE, Figure 9). The average forearm length, regardless of sex for each species, with two standard deviations extended from the mean are 32.9 – 37.5 mm (full range: 32.7-38.8 mm) for *M. brandtii* and 31.8 – 36.0 mm (full range: 31-36.9 mm) for *M. mystacinus* (Figure 9).

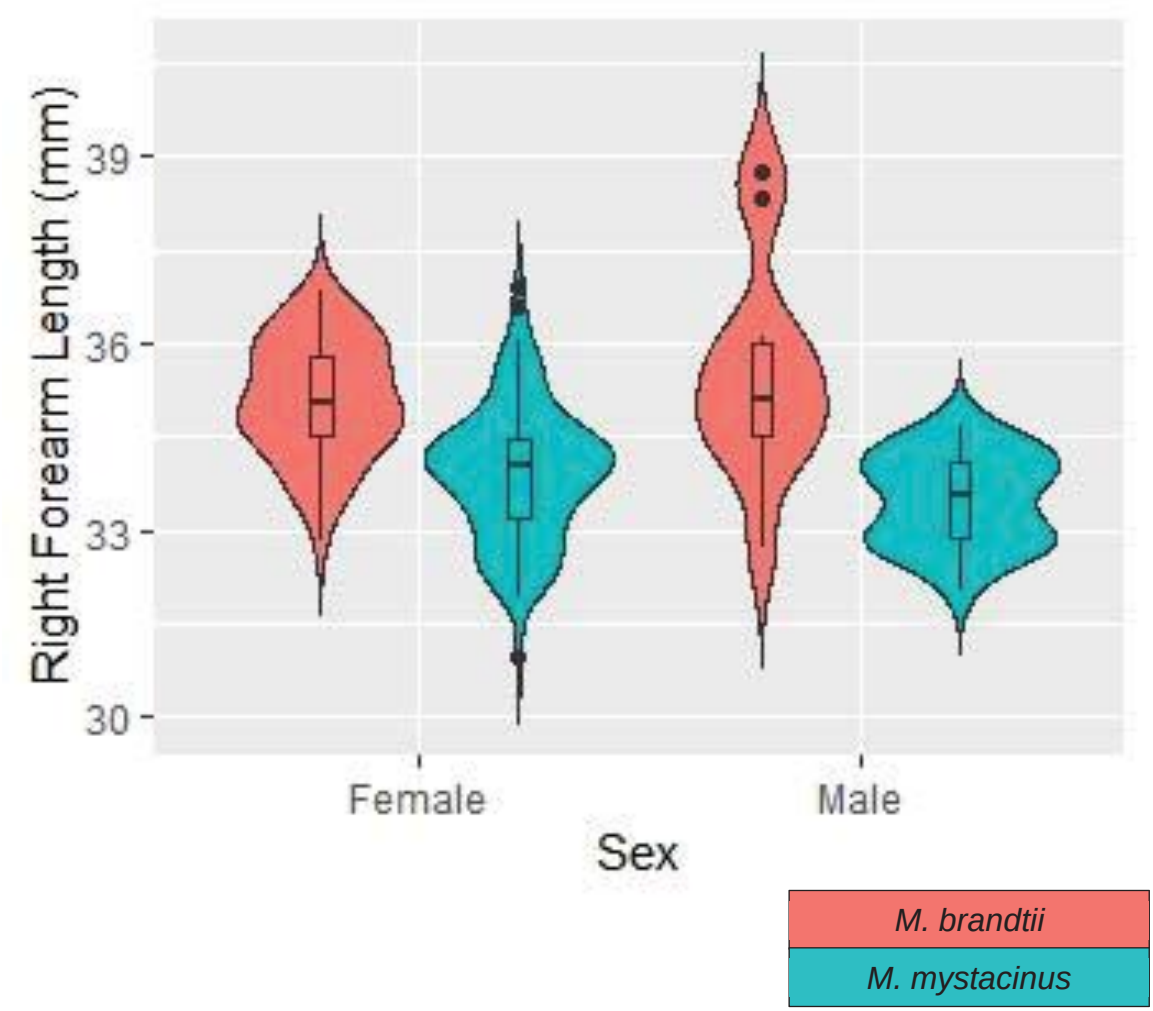


Figure 9. Violin plots with boxplots depicting the difference in body size (right forearm length) between Brandts' bats (*M. brandtii*) and Whiskered bats (*M. mystacinus*) and between male and female bats. Dots represent individual measurements that fall outside of the boxplots.

3.6 Habitat Use and Home Range Size

3.6.1 Foraging Habitat Resource Selection

For the 250 m^2 resolution RSF analysis, i.e., modelling probability of use as a function of species and the LiDAR variables zmax, zq10, zq20, zq30, zq40, zcv, and all species × LiDAR variable interactions, I found strongest support for the model including the main effects zmax, zq10, zq40, zcv and the species × zcv and species × zq40 interactions (Figure 10. a).

The best model indicated that there was a positive relationship between zmax and predicted probability of use for both species (Figure 11. a). There was also a positive influence of zcv for both species, but the relationship was relatively steeper for *M. mystacinus* (Figure11. b). There was also a positive relationship between predicted use and zq10, however the data is heavily skewed toward higher values of the 10^{th} quantile of vegetation height (Figure 11. c). The predicted use of habitat decreased with denser vegetation at the 40^{th} quantile for *M. brandtii* (Figure 11. d).

For the 16 m^2 resolution RSF analysis, starting with the same global model as for the 250 m^2 resolution, I found the strongest support for a model including only zmax and zcv as predictors, but also some support for models including one or more interactions between species and LiDAR variable(s) (Figure 10. b). The model, for which I found the strongest support, indicated that there was a positive relationship with vegetation height (zmax) and the coefficient of variation in vegetation height (zcv) and predicted use of an area regardless of species (Figure12, Table 10). Thus, in contrast to the 250 m^2 resolution, where zcv appeared to have a stronger influence over *M. mystacinus* than *M. brandtii*, no between-species difference was evident at the finer resolution.

Figure 13 illustrates the differences in predictions made by the model with the strongest support for the 250 m^2 resolution LiDAR data within the study area, by species, and then by overlaying the difference between the two species. Figure 13. d is a satellite image of the same area for comparison. This makes it possible to see that – although the two species co-occurred in the study area and showed a large degree

of spatial overlap –the species also differed in terms of predicted probability of use of the same place and habitats.

When I used a hypothesis testing modelling approach, I found a very similar final (strongest support) model for the 250m^2 spatial scale; the only difference being that the LiDAR variable zq40 was replaced with zq60 (see Appendix A1). For the 16m^2 spatial scale, the final (strongest support) model was exactly the same as when I used the information criteria approach (see Appendix A1).

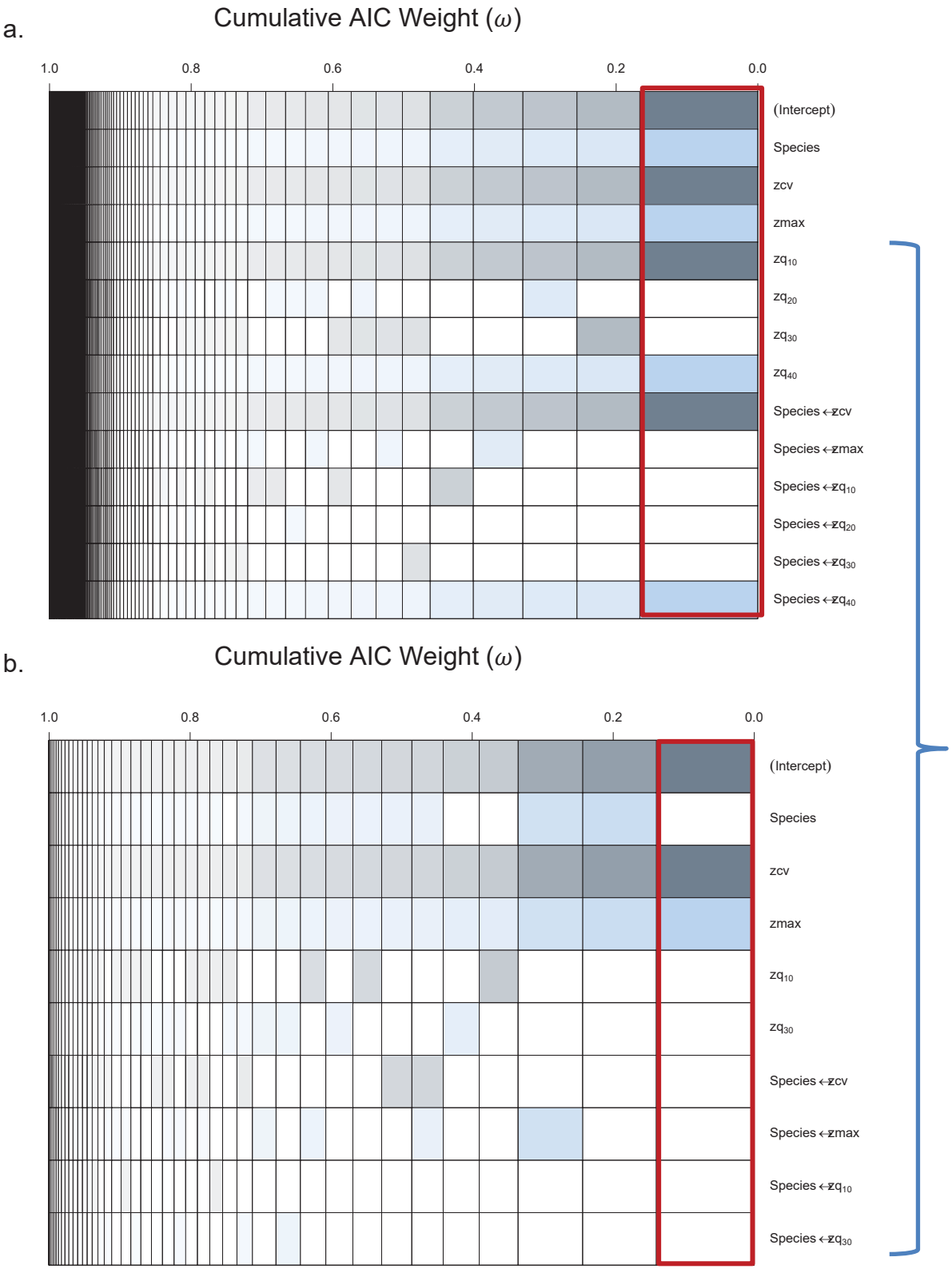


Figure 10. Fixed-effect predictor terms in the global and candidate models and cumulative AIC weights for different candidate models from the 250 m^2 (a) and 16 m^2 (b) resolutions. Fuller color reflects higher AIC weight. The red borderlines indicate the 'best' model, i.e. the model with the strongest support. Number of candidate models for which ΔAIC < 4 was 13 for analyses at both resolutions.

Table 10. Parameter estimates and associated standards errors, test statistics[1] and p-values[1] from the resource selection function (RSF) models with the strongest support (i.e. the models with the highest AIC weight), estimating probability of use as a function of LiDAR variables at spatial grains of 250 m^2 and 16 m^2. RSFs were generalized mixed models with binary response variable (Y = 1 = used, Y = 0 = available) and logit link function, assuming binomial error distribution. Input data for used habitat were LiDAR variables in tracking locations of individuals bats, for which bats were within 25 m of the observer (Y=1). Input data for available habitat were LiDAR variables for an equal number of random observations (Y = 0) generated within the 95% minimum convex polygon home range for each individual bat. Bat ID nested in species was included as random effects to account for repeated observations of the same individuals.

LiDAR variable	**β**	**SE**	**z**	**p**
Model (a): 250 m^2, R^2 = 0.73				
Intercept (*M. brandtii*)	0.130	0.078	1.673	0.094
M. mystacinus	-0.092	0.080	-1.150	0.250
Coefficient of height variation (zcv)	0.241	0.056	4.312	<0.0001
Maximum vegetation height (zmax)	0.635	0.044	14.382	<0.0001
10^{th} quantile of height (zq10)	2.951	1.757	1.680	0.093
40^{th} quantile of height (zq10)	-0.217	0.069	-3.141	0.002
Species × zcv	0.286	0.092	-3.141	0.002
Species × zq40	0.248	0.083	2.996	0.003
Random effects: Bat ID nested in Species: σ = 0.0007192, SD = 0.02682, Species: σ <0.000001, SD <0.000001				
Model (b): 16 m^2, R^2 = 0.052				
Intercept (*M. brandtii*)	-0.006	0.038	-0.172	0.864
Maximum height (zmax)	0.263	0.040	6.518	<0.0001
Coefficient of variation in height (zcv)	0.384	0.039	9.958	<0.0001
Random effects: Bat ID nested in Species: σ <0.000001, SD<0.000001, Species: σ <0.000001, SD <0.000001				

[1]I include the test statistics and p-values, even though they were not used for model selection here. This is because the AIC approach will identify the best candidate model, although all the candidate models could – in theory – be poor.

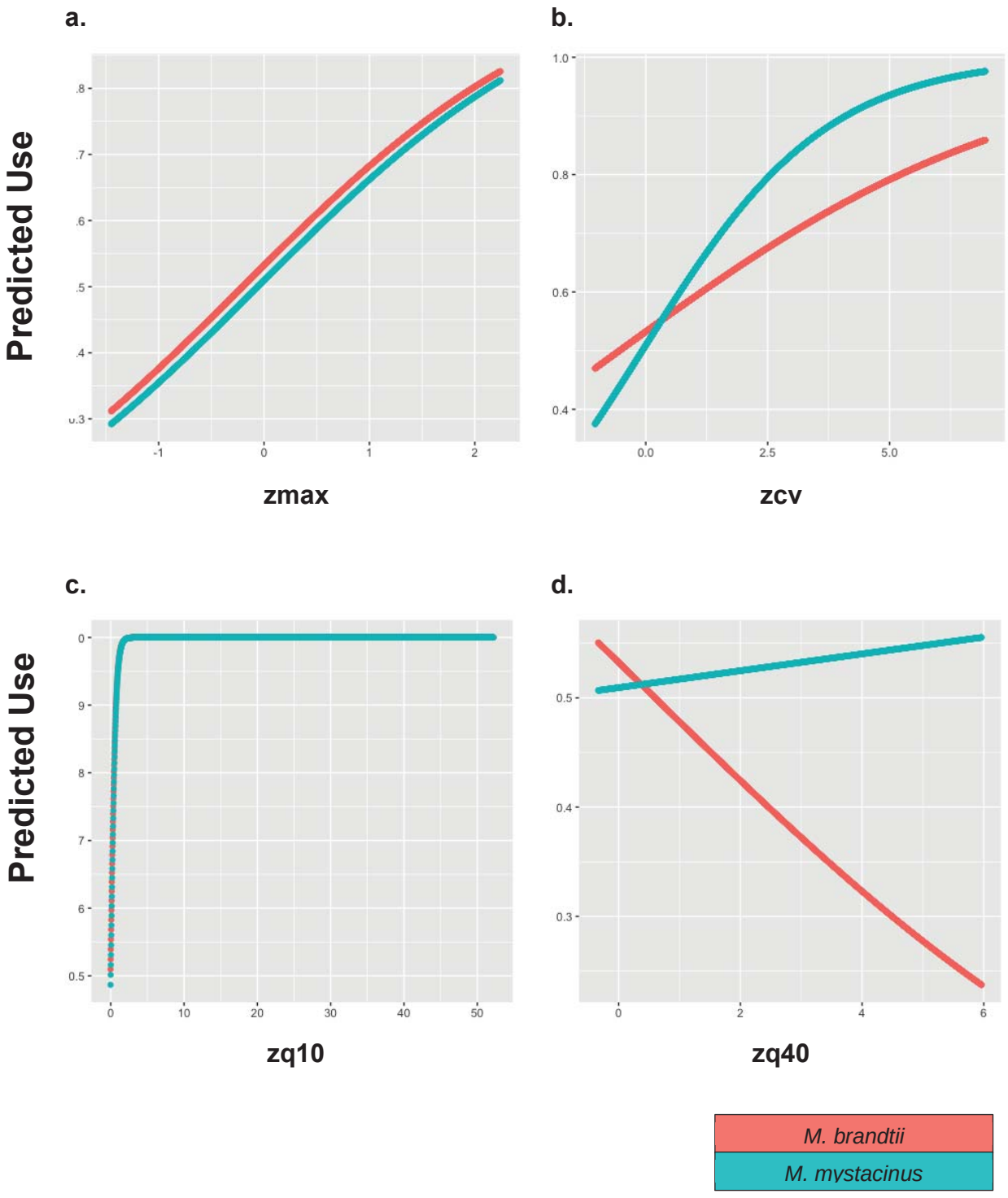


Figure 11. Predicted probability of habitat use as a function of LiDAR variables on a 250 m_2 spatial scale. Lines show predicted relationships for each of the LiDAR variables in Model (a) in Table 10, at average values of the other LiDAR variables in the model. Red and blue-green lines represent *M. brandtii* and *M. mystacinus* respectively. Note that the LiDAR variables were scaled before analyses (see Table 3).

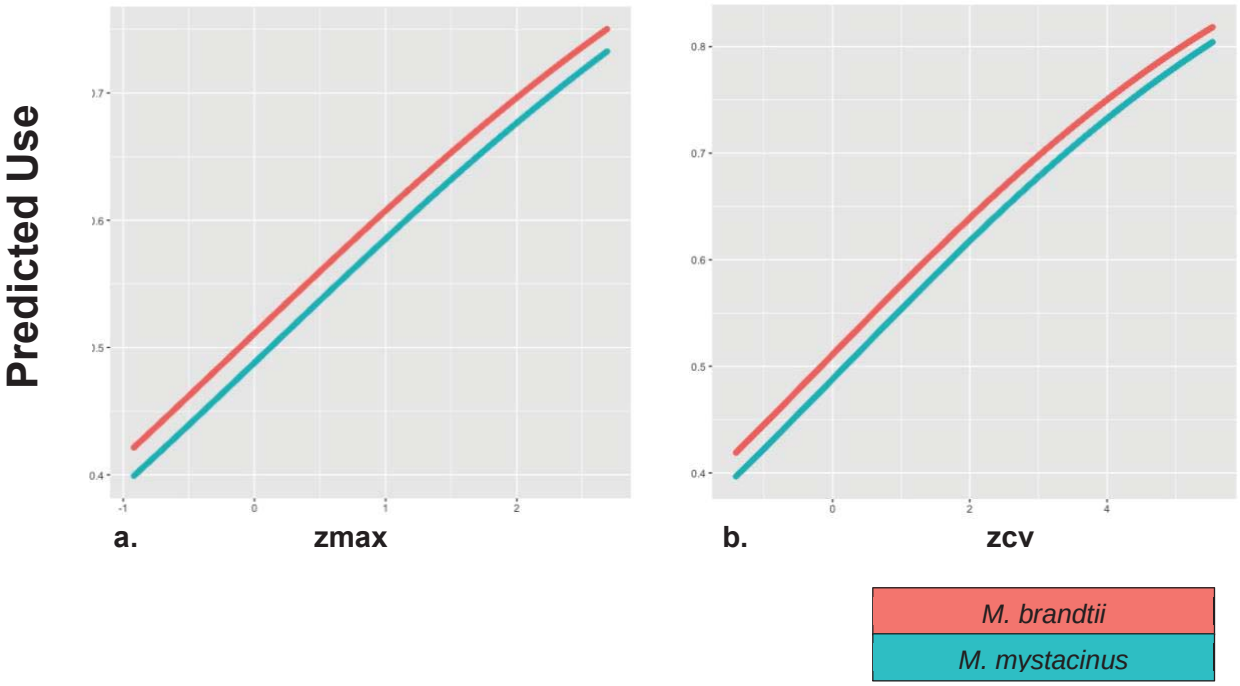


Figure 12. Predicted probability of habitat use as a function of LiDAR variables on a 16 m_2 spatial scale. Lines show predicted relationships for each of the LiDAR variables in Model (b) in Table 10, at average values of the other LiDAR variables in the model. Red and blue-green lines represent *M. brandtii* and *M. mystacinus* respectively. Note that the LiDAR variables were scaled before analyses (see Table 3).

Figure 13. a

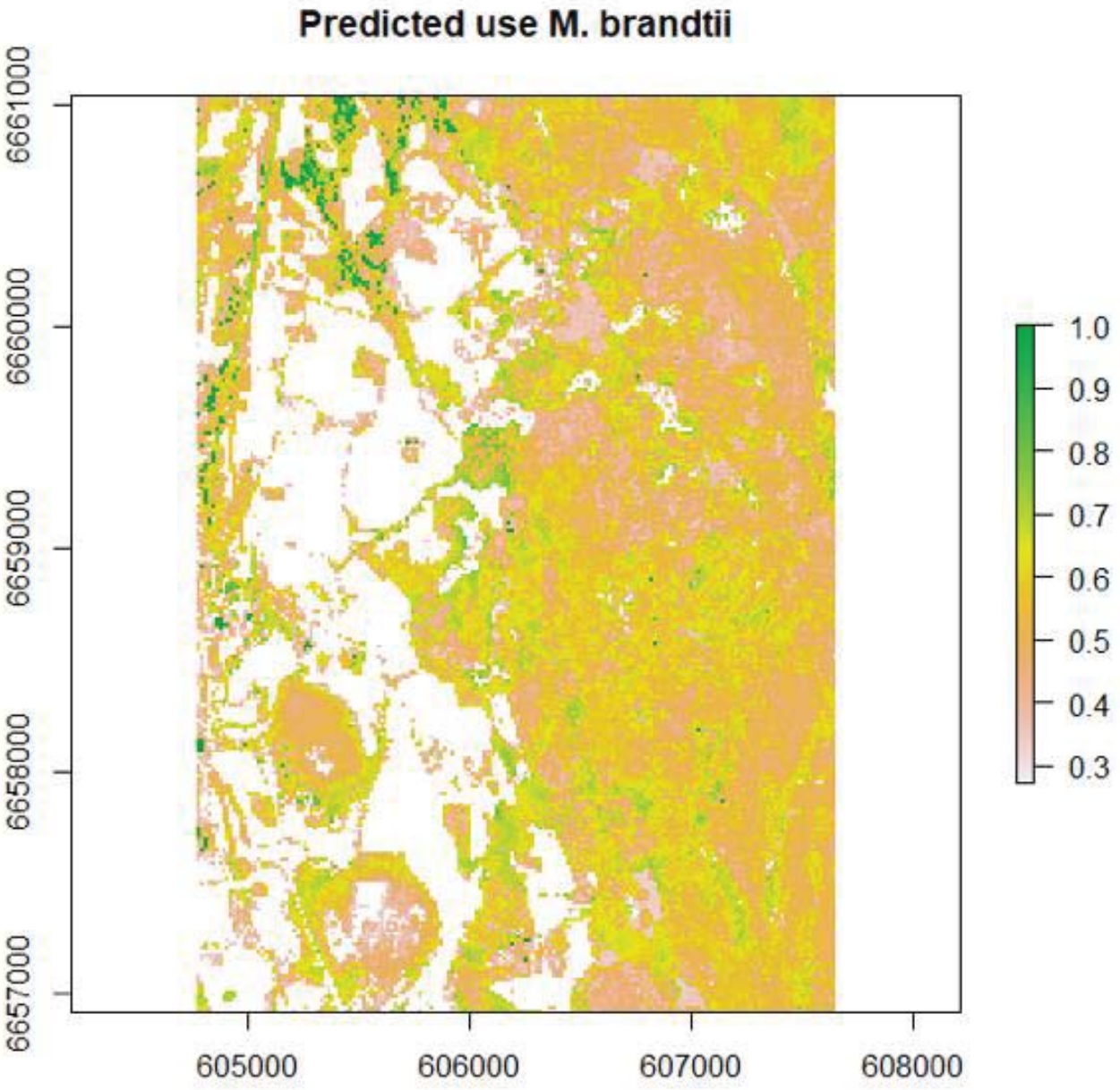

Predicted use M. brandtii
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Figure 13. b

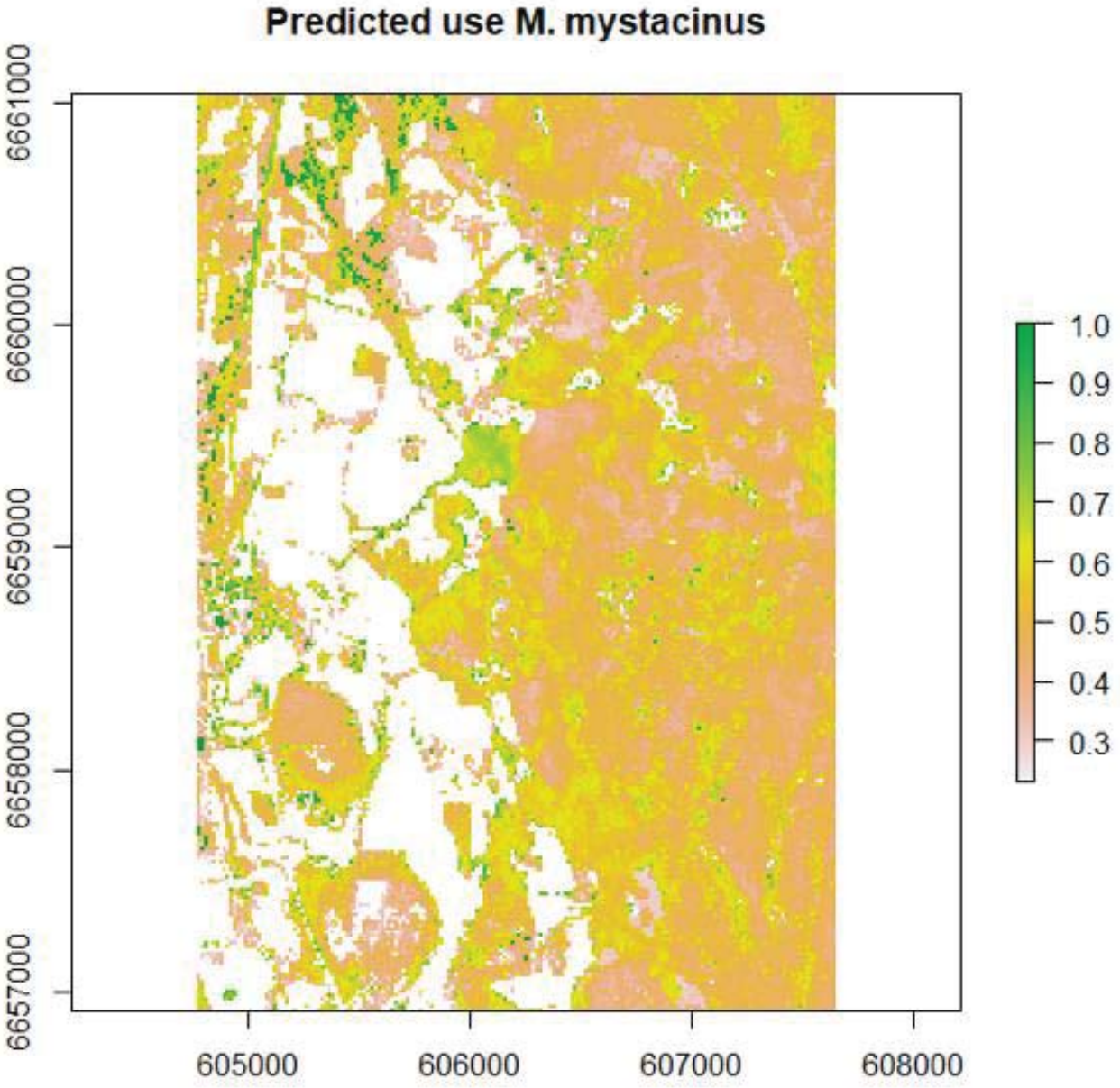
Predicted use M. mystacinus
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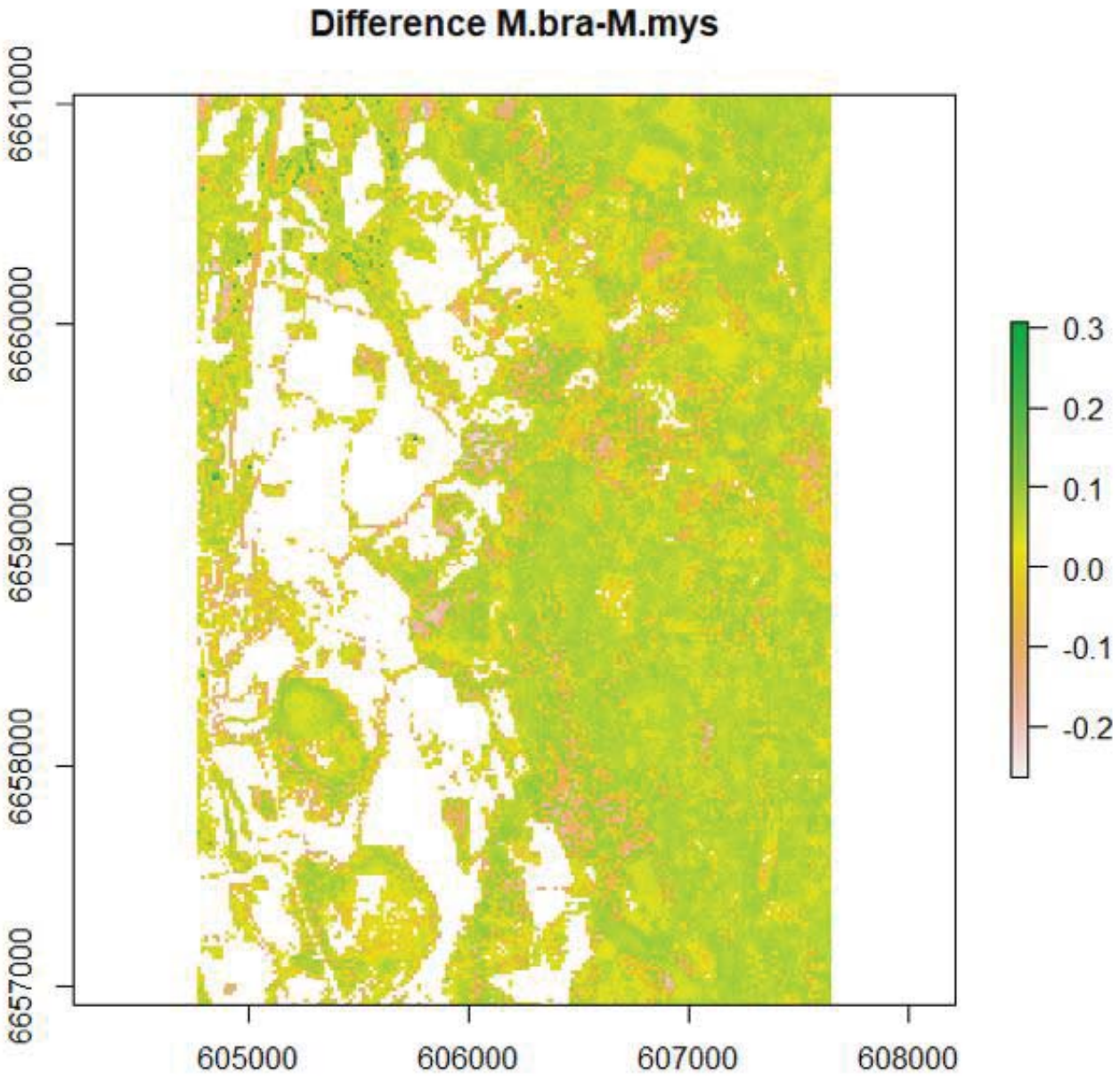
Figure 13. c


Difference M.bra-M.mys
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0.0
-0.1
-0.2

Figure 13. d

Sattelite Image of Study Area

Figure 13. Maps of the study area depicting the predicted probability of use (on a scale from 0 to 1) of habitat as determined by the LiDAR variables included in my final prediction model: zmax, zcv, zq10 and zq40 (see Table 3) for *M. brandtii* (a.), *M. mystacinus* (b.). Figure 13. c depicts the difference between the two species in the predicted probability of use of the landscape in the study area. Spatial grain of the layers (predictor variables) in the LiDAR raster stack was 250 m_2. Figure 13. d is a satellite image map of the study area retrieved from https://www.norgestkart.no.

3.6.2 Home Range Size

The average home range size as defined by 95% minimum convex polygons (MCPs) of individual bats was larger for *M. brandtii* than for *M. mystacinus* ($F_{1, 17}$ = 12.1, p=0.0029), and the influence of the number of observations per individual bat on MCP area apparently differed between the two species ($F_{1, 18}$ = 4.7, p=0.049; Figure 13). The average MCP area for each species was 8.6 km^2 and 2.4 km^2, *M. brandtii* and *M. mystacinus* respectively (Figure14).

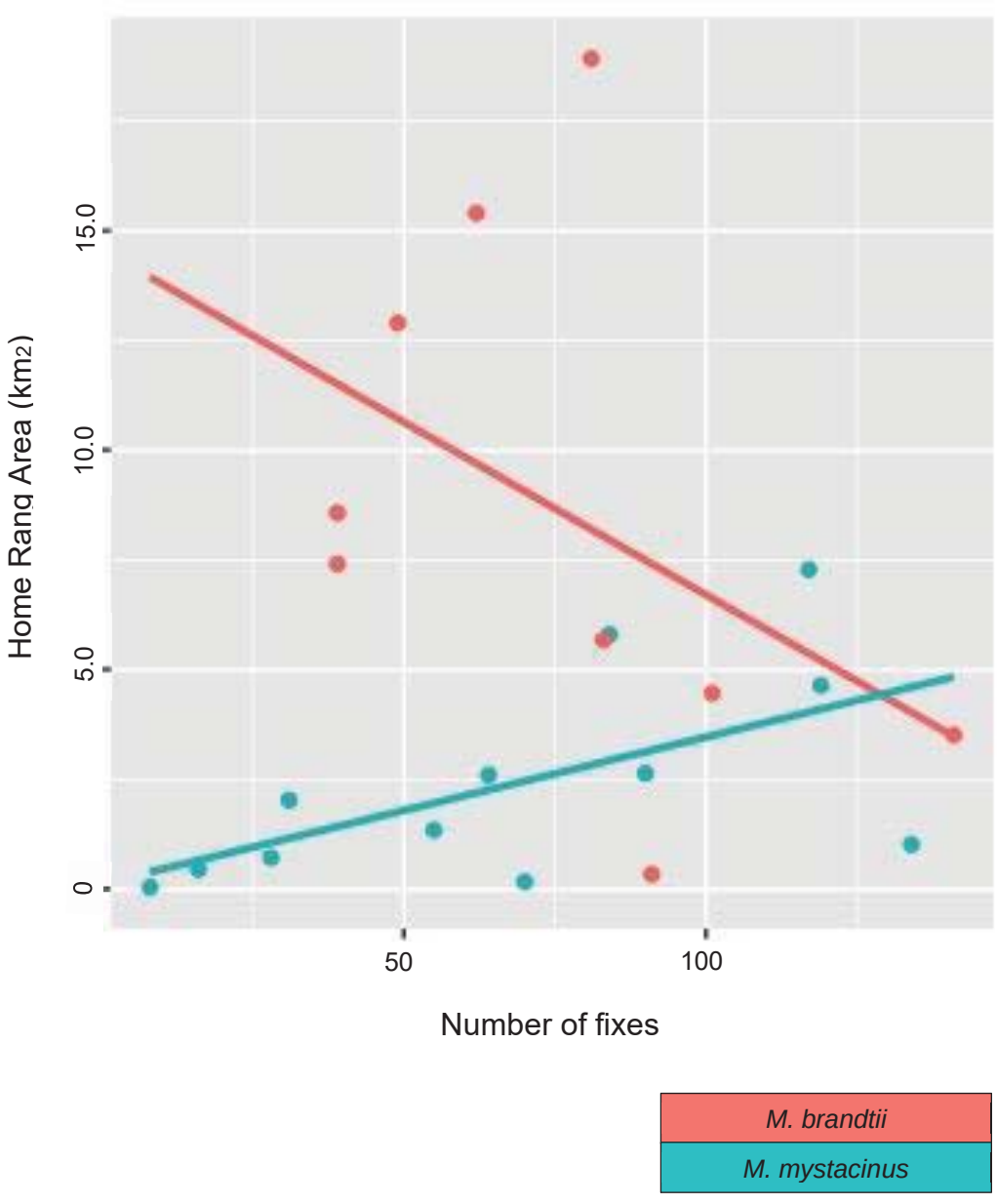

Figure 14. Scatterplot depicting the home range size area (km^2) as defined by 95% minimum convex polygons over the number of fixes (observations ≤ 25 m of a tagged bat) for each individual tagged bat by species.

4 Discussion

I found that 97.7% of the bats included in the molecular genetic analysis were correctly classified as *M. brandtii* and *M. mystacinus.* There was no significant difference in roost type selection between the two species but *M. mystacinus* were distinguished from *M. brandtii* in using rock crevices as solitary roosts. I found that the LiDAR variable describing maximum vegetation height was a strong predictor of habitat use regardless of species, spatial grain, or model building and selection approach. In this study area, we can interpret this as suggesting that forested areas were the primary foraging areas for both species. Analyses of habitat use on the coarser spatial scale at 250 m_2, revealed more nuanced differences between predicted use of habitat for each species, suggesting that *M. mystacinus* is inclined to use more heterogeneous vegetation in their foraging habitat than *M. brandtii.* There was a striking difference in home range size as defined by 95% minimum convex polygons which revealed that the *M. brandtii* in this study used an area greater than 3 times the size of *M. mystacinus.*

4.1 Identifying *M. brandtii* and *M. mystacinus*

The ranges in forearm length from the populations in this study (see Table 2) are slightly higher with more variation than those previously reported but still very closely agree with the findings of Berge (2007), Lucan et al. 2011 and Dietz and Kiefer (2016). There were two male *M. brandtii* whose right forearm measurements were exceptionally high compared to the rest of the population, at 38.4 mm and 38.8 mm in length (Figure 9). It is possible that these individuals are simply larger or that there was an error in either taking the measurement or recording it. However, these measurements only slightly fall outside the forearm length range for *M. brandtii* described by Dietz and Kiefer (2016) with an upper limit of 38.2 mm.

My results partly agree with the general trend in sexual dimorphism amongst *Vespertilionidae* with males being slightly smaller in body size than females (Myers et al. 1978, Stevens et al. 2015). When sexual dimorphism in forearm length was analyzed for each species separately, I found that males were slightly smaller than females in *M. mystacinus* but there was no significant difference in forearm length

between sexes in *M. brandtii.* It is important to note that males made a up much smaller portion of the bats captured for both species. Also, two male *M. brandtii* had right forearm measurements that were exceptionally long in relation to the rest of the sample. Such outliers may be due to true exceptions in the population otherwise they are due to errors in taking measurements, species determination or recording data.

By using dentition and penis shape as primary diagnostic features and taking into consideration forearm size, pelage color, tragus shape and thumb-claw lengths as secondary diagnostic features, we were able to distinguish between *M. brandtii* and *M. mystacinus* with 97.7% accuracy. There was an individual *M. mystacinus* which was erroneously classified as *M. brandtii* in the field.

Teeth were the primary diagnostic feature most often used for species determinations in the field between *M. brandtii* and *M. mystacinus* in this study. Teeth can be worn down or damaged over time in a way that makes it difficult or impossible to see the distinctive protocone that extends off of P_4 in *M. brandtii* and in such cases a bat may appear to be *M. mystacinus* superficially (von Helversen et al. 2001) Furthermore, there can be individual variation in size and shape of teeth, as well as asymmetric dental characteristics that further complicates using this feature as a diagnostic tool. Dentition should be taken into consideration with other diagnostic features such as forearm length, pelage color, and tragus shape in order to improve the likelihood of making a correct species designation. Whenever it is feasible to do so, genetic verification of in hand identification between *M. mystacinus* and *M. brandtii* is ideal. This is especially true if the field crew is not experienced in differentiating between cryptic *Myotis* species or when working with maternity colony populations. Maternity colonies primarily consist of adult females and sub-adults. Such that, the penis shape feature is not of use or cannot be relied upon and some features may have not fully developed in the subadults.

I have been able to find only two previous studies which have used molecular genetic analysis to test in hand identification of *M. brandtii* and *M. mystacinus.* Berge (2007) used multivariate as well as univariate analyses to determine which diagnostic features best differentiated the two species. Berge was able to claim a 100% rate of success in distinguishing between *M. brandtii* (n=65) and *M. mystacinus* (n=77) when

taking into consideration a combination of features: dentition from the maxilla and the mandible, penis shape, tragus shape and thumb claw length but concluded that due to the amount of overlap in any one of these features, in-hand field identification still carries the risk of misidentification. Lucan et al. (2011) compared the in-hand classification success via molecular genetic analysis of determining between *M. brandtii, M. mystacinus,* and *M. alcathoe (*n=359 total) in the Czech Republic and was able to claim a 96.1% rate of accuracy in distinguishing between these cryptic species using the forearm length, body mass, and tibia length to make these distinctions. Berge (2007) and Lucan et al. (2001) used wing biopsies to extract bat DNA whereas I chose to use feces, which can be a less reliable source of DNA but is much less invasive. Through the molecular genetic analysis of feces from capture sites, I am able to say with a high degree of confidence that the *M. brandtii* and *M. mystacinus* in this study were identified to species level correctly despite their cryptic morphology.

Regardless of experience, misidentification for a number of bat species, especially within the *Myotis* genus, remains a possibility. In this study, there was not a standardized method for recording which features were included in species determination for each individual and only photos of wings were taken of each bat. It is my recommendation that in future studies, whenever there is a risk of mistaking one species for another, to take photos as evidence of diagnostic features for each individual bat in order to better account for human error. In the case of *M. brandtii* and *M. mystacinus* this should include a photo of the profile of the bat so that its ears, tragus, face shape and pelage are all clearly visible as well as photos of the profile dentition on both the right and left side of the bat. In circumstances where there is risk of misidentifying these two species for *M. daubentonii,* one might also include photos of a foot of the bat with the uropatagium extended such that is clearly visible where the membrane attaches, and toe hairs are visible. These photos may expose the animals to some extra stress from extended handling but can be an otherwise non-invasive method of documenting morphological differences between individuals and species.

4.3 Foraging Habitat, Roost Habitat and Home Range Size

I found that both species were predicted to select areas with higher vegetation and with a higher degree of heterogeneity (coefficient of variation) in vegetation height regardless of the spatial grain of the LiDAR data. In this study area, this suggests that both species are highly selective of forests. The variability of the vegetation height was of more importance for *M. mystacinus* than to *M. brandtii* at the coarser spatial grain of 250 m^2 as compared to the 16 m^2 grain. Furthermore, the LiDAR variables describing the 10^{th} and 40^{th} quantiles of vegetation height (zq10, zq40) were only significant predictors of habitat selection at the 250 m^2 grain. There was a weak positive relationship for predicted use of an area with zq40 for *M. mystacinus* while there was a stronger negative relationship for *M. brandtii.* The final model for the 250 m^2 resolution explained 73% of the variation in the data while the final model 16 m^2 resolution only explained 5.2%. This suggests that at a coarser scale, it is possible to see that *M. mystacinus* tend to use more varied habitat in terms of vegetation structure with a denser middle story than *M. brandtii.* It is also within reason to suggest that as *M. mystacinus* is more closely associated with varied and denser vegetation, that they may be utilizing non-forested areas more so than *M. brandtii* such as grasslands and other open landscapes.

Previous research on the foraging habitat of *M. brandtii* and *M. mystacinus* have found evidence of variation between the two species but the results as well as the methodologies, spatial scale and regions where these studies took place vary. Taake (1984) used the area surrounding maternity colony locations in Germany as a proxy for foraging habitat and concluded that *M. mystacinus* is more likely to use anthropogenic landscapes such as pastures and villages while *M. brandtii* was more associated with woodlands, especially deciduous forests near streams. Berge (2007) claims to be the first to compare the foraging habitat of *M. brandtii* and *M. mystacinus* using radio telemetry while also verifying species classification using molecular genetic analysis. They studied several colonies of both species from southern U.K. and found similar results suggesting that *M. brandtii* were more likely to use forested areas than *M. mystacinus* but with coniferous forest being preferred over deciduous while *M. mystacinus* was associated with grasslands. Berge (2007)'s study took place in Britain and used homing in as well as triangulation to make observations of foraging

bats. However, no tests were utilized such as those in my study to determine the proximity of the bat to the observer and so there is a higher degree of uncertainty for bat foraging locations. Berge's study took place in a region with much darker and longer summer nights than my own study area which also sets it apart from my study. Furthermore, habitat was described in Berge (2007) using categorical landscape descriptions from satellite images and ground level habitat surveys. Buckley et al. (2013) used radio telemetry in combination with similar categorical habitat descriptions to study the foraging habitat selection of *M. mystacinus* in Ireland. They observed *M. mystacinus* using a variety of foraging habitat including forests and grassland as well as agricultural areas but distinguished that only mixed forests and riparian areas were used as core foraging areas – contradicting Berge (2007)'s findings. Wickramasinghe et al. (2003) found that *M. brandtii* is frequently found foraging at organic farms in the U.K., which provides some evidence that this species is not exclusively forest foraging.

Roswag et al. (2019) used stable isotope analysis of hair from and wing biopsies of *M. alcathoe, M. brandtii* and *M. mystacinus* in Germany to compare habitat between these cryptic species, however the sample size for *M. mystacinus* was limited and excluded from most analyses. This study found that *M. mystacinus* and *M. brandtii* were also very similar, but with *M. mystacinus* using the broadest range of habitat of all three species while *M. brandtii* was primarily associated with forests. Wermundsen and Siivonen (2008) used acoustic walking transects to describe the foraging behavior of common bat species in Finland, which included grouping together *M. brandtii* and *M. mystacinus,* and found that these species were almost exclusively found foraging in forest habitat. A bachelors' thesis from Sweden examining how *M. brandtii* and *M. mystacinus* foraged near roads and railways observed both species using villages, forest as well as riparian areas for foraging habitat (Kammonen 2015). In this study, bats were radio-tagged and homed in on, but the focus was on the bats foraging in relation to a roadway and the distance between the observer and the bat was presumed rather than tested. Furthermore, no genetic analysis was included to verify in-hand identification of *M. brandtii* and *M. mystacinus.* These studies are close in proximity to my own in terms of time and geographic location but there lacks good representation for both species and/or precise methodologies that allow the researcher to distinguish between the two species confidently. All together, these studies demonstrate that the habitat selection of *M. brandtii* and *M. mystacinus* varies

and there is a need for further exploring evidence of resource partitioning between the two in their foraging habitat selection, especially at the northern end of their range.

M. brandtii and *M. mystacinus* have ranges that span across the British Isles and continental Europe (Dietz and Kiefer 2016) where they can occupy a large array of landscapes with varying degrees of availability to different habitat types. Furthermore, the way in which different habitat types are classified can vary between regions and studies. It is also important to note that while the two species often occupy the same areas, there are areas where they do not coincide, such that the results of studying one species in the absence of the other may also reveal how different ecological pressures may influence their resource selection. For example, *M. brandtii* is rarely found in Ireland and furthermore Ireland does not have much continuous forest (Boston et al. 2010). Thus, comparing the foraging behavior of *M. mystacinus* reported by Buckley et al. (2013) to my own study area in southeastern Norway where both species are found in the same valley with abundant access to forests is not a clear parallel. Exploring the ecological impact of variables such as forest connectivity and heterogeneity are more applicable to understanding ecological dynamics on a global scale, so as to avoid conflicting definitions of categorical landscape definitions and make it possible to compare studies from different landscapes across time and space.

Within my own dataset, there was a great deal of variation in number of observations per individual, but I have accounted for this by including bat ID as a random effect in my analyses. RSF and MCP analyses both found that the interaction between the number of observations and species had a significant influence in the models, though this may be more relevant to home range than habitat use. Furthermore, we had a large field crew to meet the amount of effort needed to collect this data, and individual variation in recording observations can also introduce some extra ‘noise’ in the data. Until it is feasible to use satellite/GPS telemetry tags on small bat species, foraging ecology studies based on radio-telemetry surveys of small bat species will continue to struggle with these types of precision errors. Even so, this type of data is valuable and worth pursuing creatively with the tools currently available. This study shows that homing in can be a viable technique for tracking foraging bats under some circumstances and especially at northern latitudes where bats experience extremely shortened night periods, with extended twilights which make bats more visible to

observers. This is practical for studying maternity colony populations at northern latitudes, whose foraging ranges may be more restricted in order to compensate for the lack of time available to hunt while meeting high energy demands.

To my knowledge, this study is the first to relate high precision foraging observations of *M. brandtii* and *M. mystacinus* to LiDAR data and to use a resource selection analysis to compare their habitat use. Jung et al. (2012) and Froiedevaux et al. (2016) related foraging habitat of bats in Switzerland and North-east Germany respectively, through stationary passive acoustic monitoring and vegetation LiDAR data. These studies provided valuable insights on which LiDAR variables were important to include with respect to habitat use of *M. brandtii* and *M. mystacinus.* However, due to the fact that *M. mystacinus* and *M. brandtii,* in addition to most European *Myotis* species, cannot be reliably distinguished from one another using their acoustic monitoring techniques (Vaughan et al. 1997b), it is difficult for me to draw comparisons between the ecological results of this study and my own. My findings agree with Froiedevaux et al. (2016) and Jung et al. (2012) that forested areas with varied canopy structure are important foraging sites for *Myotis* species.

In ecology, it is vital to understand at what scale a process is happening, but the answer may not lend itself inherently (McGill 2010, Henle et al. 2014),Most studies that have explored bat foraging ecology have done so in relation to habitat descriptions that are categorical and that primarily take into consideration 2-dimensional characteristics. For many ecological questions, this could be adequate but due to the fact that bats rely more so on 3-dimensional space, with the vertical dimension playing an vital role in their behavior, it is important to find methods for relating this in ecological studies. As LiDAR has become increasingly accessible to wildlife researchers, there have been many studies that use it to describe the continuous and 3-dimensional habitat of birds (Rothery et al. 2009, Bakx et al. 2019, Eldegard et al. 2014, Melin et al. 2018). Compared to birds and larger non-volant mammals, LiDAR has been used in far fewer cases to describe bat habitat (Vierling et al. 2008). LiDAR has been used to describe bat roost sites in caves (Azmy et al. 2011, Shazali et al. 2017) and has been used in relation to bat acoustic activity (Jung et al. 2012, Froidevaux et al. 2016, Müller et al. 2013).

Given that collecting data on foraging bats, especially small insectivorous species, can be so challenging, it is also difficult to find opportunities to test using LiDAR as a means of describing land resources for bats. Here we tested two different LiDAR resolutions to account for variations in scale and the coarser scale of 250 m^2 provided more nuanced results which support other evidence that *M. mystacinus* is more generalized in habitat use than *M. brandtii.* It is possible that the 16 m^2 resolution is too fine to distinguish anything meaningful from these species,

The 250 m^2 resolution, though coarser, is still considered high resolution in describing forested habitat (habitat (e.g. the Norwegian National Forest Inventory measure forest attribute variables in core plots of size 250 m^2; Viken 2017, Chopping et al. 2009). This resolution has been highly effective at describing forested landscapes in combination with other landscape description tools such as MODIS or Landsat (Hudak et al. 2002, Li et al. 2015). The approach that I have used in this study did not make it possible to explore when bats were using different habitat types such as riparian areas or grasslands clearly but using a more sophisticated approach in combination with other landscape description tools would be helpful in this case. Given the novelty of this study, future research should continue to experiment with different scales of LiDAR resolution in order to best understand what resolutions and land description variables are most relevant to bat ecology. Furthermore, composite measures of vegetation structure (such as the coefficient of variation variable included in this study) should be explored in order to describe habitat features which are of importance to bat species. My study demonstrates LiDAR is capable of illustrating meaningful differences in habitat selection for bats and represents an alternative to categorical and 2-dimensional descriptions of habitat.

I found no significant differences in terms of roost selection or colony size for the two species however it is interesting that *M. mystacinus* was observed using rock crevices on several occasions during the maternity season whereas *M. brandtii* was never observed roosting in rock crevices. This may further suggest that *M. mystacinus* is a more generalist species than *M. brandtii.* Overall, my findings agree with Berge (2007), Dietz and Kiefer 2016), Nyholm (1965) and Taake (1984), that both M. *brandtii* and *M. mystacinus* are frequently found in anthropogenic structures such as houses, barns and outbuildings. It is important to note that my data is also collected from a population

of bats that are mostly associated with one colony for each species in close proximity to each other. There remains a need to further study these two species in relation to one another in other regions of Norway and throughout their northern range. For example, even other populations local to those studied here provide different results.

The substantial difference in home range size between the two species may be a result of *M. brandtii* commuting farther distances to find suitable foraging habitat whereas *M. mystacinus* may have been better suited to use the cultural landscapes and less mature forests more readily available around the main colony sites, which were in residential homes. Berge (2007) and Buckley et al. (2013) used similar techniques for tracking bats and defining home range size to my own methods. Berge (2007) found that *M. brandtii* travelled farther distances for foraging than *M. mystacinus,* but that the average home range size for the two species was nearly the same (.43 and .41 km^2 for *M. brandtii* and *M. mystacinus* respectively). Buckley et al. (2013) found some variation home range size for *M. mystacinus* in Ireland, ranging from 1 to 8 km^2, similar to the home ranges of *M. mystacinus* from my study which ranged from 0.05 to 7.2 km^2. Buckley et al. included roosts within their estimations of home range sizes, whereas I chose not to, which likely accounts for differences in our minimum home ranges.

Regional differences in studies as well as variations in tracking methods, number of observations, days spent tracking, and temporal distinctions in study efforts all play important roles in the outcomes of calculating home range. The shortened night periods experienced by the bats in my study likely also influence their home range size and foraging site fidelity more so than bats at southern latitudes that have more time to forage. Dense and Rahmel (2002) observed that adult female *M. brandtii* in northern Germany during the maternity season will forage the whole night regardless of weather conditions. It may be that bats are northern latitudes that experience very brief night periods must compensate for lost time by foraging intensively in this way. Further research that use high precision foraging telemetry throughout the range of these species – and in different landscape contexts – would be highly beneficial in better understanding the distinctions in home range size between these two species.

4.4 Diet

Due to the COVID-19 pandemic, the final results for arthropod composition in the diet of *M. brandtii* and *M. mystacinus* from the molecular genetic analysis of feces have been delayed in their delivery. In lieu of their absence I have compiled a brief literature review to discuss in the context of my other results.

Berge (2007) used micro histological methods for examining the feces from maternity colonies of *M. mystacinus* and *M. brandtii* and found that there were significant differences as well as seasonal variations in the diet between the two species. Insect remains found in the feces primarily belonged to *Diptera* and *Lepidoptera* with a great deal of variation in the genera represented. *M. brandtii* had a diet composed more so of insects associated with aquatic areas whereas *M. mystacinus* had a diet with insects more often found in grasslands and pasture. Furthermore, there was a large representation of diurnal and nonvolant insects which would suggest gleaning behavior for both species. Berge also discovered some temporal variation in the diet of both species, with the highest diet diversity found in fecal samples collected in the middle of the summer. These results are similar to those found by Hollyfield (1993) which reported *M. mystacinus* in same region of England as having the most diverse diet during June and July. Berge concluded that the trends found in the diet of the bats included in their study are most likely due to variation in prey abundance throughout the season as well as variation in habitat selection, but that further research is necessary to explore these hypotheses.

Andres (1995) also used micro histological methods to examine the feces of several bat species in from throughout Switzerland and Germany and found that *M. mystacinus* often preyed on arthropods that were either diurnal or incapable of flight, providing evidence that these bats glean prey off of vegetation.

Vesterinen et al. (2018) used techniques for genetic metabarcoding of feces closely resembling those used in this study and with the same study species on bat populations in southern Finland. The sample size for feces for *M. mystacinus* was too small to include in statistical analyses in this study but they were able to report that for both *M. brandtii* and *M. mystacinus, Lepidoptera* made up the most dominant portion

of their diet. This study found no significant temporal variation in diet across the five bat species investigated (*M. brandtii, M. daubentonii, M. mystacinus, E. nilssonii,* and *P. auritus*), contrary to Berge's findings. Roswag et al. (2019) also used molecular fecal analysis of *M. alcathoe, M. brandtii* and *M. mystacinus* to compare diet within and between the species. This study found that *Coleoptera, Diptera* and *Lepidoptera* made up 100% of the diet of all three bat species and that the diet of *M. brandtii* and *M. mystacinus* were especially similar.

Bats are often classified into guilds or ecomorphs to describe their different foraging strategies, including diet as well as behavior related to flight and echolocation (Denzinger et al. 2013, Ghazali et al. 2016). There is a great deal of variation on how to define these guilds and which species might belong to them, but in general *M. brandtii* and *M. mystacinus* are frequently categorized as some variation of edge-space aerial foragers (Dietz and Kiefer 2016, Ghazali et al. 2016, Müller et al. 2013).

Given that there are special constraints placed on bats at higher latitudes in terms of reduced darkness / shortened night periods and a cooler climate, it would be interesting to see if there are deviations from the strategies observed from southern populations from more central Europe of *M. brandtii* and *M. mystacinus.* Boyles et al. (2016) found that *M. lucifugus* in Alaska foraged for shorter periods of time but with a higher 'refueling rate' (determined by measuring plasma-ß-hydroxybutyrate concentrations from blood samples) than conspecifics at southern latitudes. Boyles et al. (2016) also found that *M. lucifugus* from their study foraged on spiders, which would require gleaning in cluttered forest rather than forest edge space where this bat species is typically found foraging otherwise. There is some other evidence that bats at northern latitudes have longer torpor bouts in the summer to compensate for the high energy demands in addition to foraging more extensively on small, aerial prey (Rydell et al. 1992). Increasing our understanding of the diet of *M. brandtii* and *M. mystacinus* in relation to each other and their foraging habitat, will shed light on the broad ecological context they exist in and inform management of the species.

The range of *M. mystacinus* in Norway is more extensive than *M. brandtii,* which is restricted to the southeastern portion of the country. The results of the dietary analyses may reveal interesting associations between forest or habitat types found in these

different regions relevant to the two species based off of which insects were more prevalent in their feces. Collecting feces from net sites is a relatively non-invasive measure that can potentially reveal a great deal of information about the habitat selection, physiology and diet of bat species. Though I cannot report on dietary results at this time, I can confidently recommend that further studies include this method whenever it is feasible.

4.4 Management Implications

Bat maternity colonies are especially ecologically sensitive places that should be prioritized for research and protection (Sheffield et al. 1992). Describing and understanding the basic ecological roles of species, especially with regards to maternity colonies is a primary role of wildlife managers. To protect these species when they are most vulnerable it is necessary to study the differences in habitat selection as well as to identify important foraging and roosting sites in order to make evidence-based management decisions (Mickleburgh et al. 2002). Although *M. brandtii* and *M. mystacinus* are not red-listed species or considered threatened in Norway, they may be in the future, at which point identifying and protecting colony roost sites as well as foraging habitat should be emphasized. Furthermore, the methods used in this study may be applied to studying other bat species elsewhere in the world for a variety of purposes.

5 Conclusion

As there remains some possibilty to mistake these two species in the hand for one another, I suggest that genetic analysis should be included in studies where distinguishing between these two species is important, especially when studying maternity colony populations. *M. brandtii* used a foraging area over 3 times the size of M. *mystacinus*, though both species used similar roosts and colonies in close proximity to one another. I have found that using LiDAR data to describe the continuous, 3-dimensional characteristics of habitat can reveal important distinctions in foraging habitat selection between these two species. My findings further support previous research which suggests that *M. brandtii* are strongly associated with forests while *M. mystacinus* is more generalized in habitat use while both species are primarily associated with foraging in forest. I was able to determine that variability in canopy structure and canopy height were important features in foraging habitat for both species.

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Appendix

A1: Hypothesis Test Approach Resource Selection Function

For both *M. mystacinus* and *M. brandtii*, many of the LiDAR variables influenced the predicted probability of habitat use when included as single explanatory terms, for both spatial resolutions of the LiDAR variables (Table A1). For each bat species, LiDAR variable and spatial resolution combination, I fitted generalized mixed models with binary response variable (Y = 1 = used, Y = 0 = available) and logit link function, assuming binomial error distribution. Bat ID was included as a random effect. Many of the LiDAR variables were strongly correlated (see Table A2, Table A3) and could therefore not be included as explanatory variables in the same statistical models.

For each species, I selected variables with $p<0.001$ for inclusion in the full (most complex model). If two or more variables were strongly correlated ($r>0.60$), only the variable with the strongest signal on the response (lowest p-value) was included in the model. Finally, LiDAR variables from the species-specific models were combined in the same model, again using the same criteria for avoiding collinearity. The full (most complex) RSF model, the selected LiDAR variables, species and the interactions between species and each of the selected LiDAR variables were included as fixed effects, and Bat ID nested in species was included as random effects. Model selection was carried out by backward elimination, sequentially dropping non-significant terms ($p<0.05$) using a likelihood ratio test. The final RSF model only included significant terms.

Equation (1) refers to the 16 m^2 resolution final model and equation (2) for the 250 m^2 resolution:

(1) glmer(used ~ species + zmax + zcv + (1|speces/bat ID)

(2) glmer(used ~ species + zmax + zcv + zq60 + species:zcv + species:zq60 + (1|species/bat ID)

When analyzing both species together at the 250 m_2 resolution, I found that the predicted probability of use was positively related to maximum height of the vegetation (zmax) for both species, there was no difference between the species with respect to the influence of maximum vegetation height (p=0.09); Table A4; Figure A1.a). For both species, there was also a positive relationship between predicted habitat use and the coefficient of variation in vegetation height (zcv), but this positive relationship was significantly stronger in *M. mystacinus* than in *M. brandtii* (Table A4, Figure A1.b). Also, there was a strong positive influence of the 60_{th} vegetation height quantile (zq60) for *M. mystacinus*, but not for *M. brandtii* (Figure A1.c). At the 16 m_2 resolution of the LiDAR variables, I found that the probability of use increased with maximum height of the vegetation (zmax) and of the coefficient of variation of the vegetation height (zcv), but there was no significant differences between the two species (zmax: p=0.15, zcv: p=0.45); Table A4; Figure A1.d, Figure A1.e).

Table A1. Relationships between estimated probability of use and each of the LiDAR variables in single-variable models with a binary response variable (Y = 1 = used, Y = 0 = available), for LiDAR variables extracted at spatial resolutions of 250 m_2 and a finer grid of 16m_2. Input data for used and available observations were tracking locations of individuals bats, for which bats were within 25 m of the observer (Y=1) and an equal number of random observations (Y = 0) generated within the 95% minimum convex polygon home range for each individual bat. Bat ID nested in species was included as random effects to account repeated observations of the same individuals.

LiDAR variable	*M. mystacinus* (n = 12 individuals)				*M. Brandtii* (n = 9 individuals)			
	β	SE	z	p	β	SE	z	p
250 m_2								
Intercept	-0.0012	0.0495	-0.0250	0.9800	-0.0073	0.0541	-0.1350	0.8920
zmax	0.5685	0.0568	10.0120	**<0.0001**	0.5987	0.0575	10.4060	**<0.0001**
zmean	0.2896	0.0493	5.8810	**<0.0001**	0.3377	0.0634	5.3280	**<0.0001**
zsd	0.3737	0.0508	7.3580	**<0.0001**	0.4943	0.0614	8.0470	**<0.0001**
zcv	0.3640	0.0634	5.7400	**<0.0001**	0.2823	0.0526	5.3670	**<0.0001**
pzabovezmean	-0.1050	0.0500	-2.0990	0.0358	-0.4278	0.0567	-7.5510	**<0.0001**
pzabove0.5	0.2335	0.0502	4.6490	**<0.0001**	0.3393	0.0588	5.7750	**<0.0001**
zq10	1.0865	28.0722	0.0390	0.9690	2.5518	1.6887	1.5110	0.1310
zq20	0.0676	0.0821	0.8230	0.4100	0.1397	0.0699	1.9990	0.0456
zq30	0.0622	0.0483	1.2870	0.1980	0.0915	0.0608	1.5050	0.1320
zq40	0.1130	0.0462	2.4470	0.0144	0.0338	0.0621	0.5440	0.5860
zq50	0.2034	0.0469	4.3400	**<0.0001**	0.0820	0.0627	1.3070	0.1910
zq60	0.2637	0.0474	5.5590	**<0.0001**	0.1801	0.0637	2.8280	0.0047
zq70	0.2648	0.0482	5.4950	**<0.0001**	0.3133	0.0632	4.9610	**<0.0001**
zq80	0.2716	0.0491	5.5310	**<0.0001**	0.3601	0.0619	5.8210	**<0.0001**
zq90	0.2935	0.0500	5.8720	**<0.0001**	0.4001	0.0604	6.6220	**<0.0001**
16 m_2								
Intercept	0.0074	0.4963	0.1490	0.8820	-0.0297	0.0545	-0.5450	0.5860
zmax	0.3763	0.0514	7.3190	**<0.0001**	0.2973	0.0574	5.1820	**<0.0001**
zmean	0.2612	0.0499	5.2320	**<0.0001**	0.2208	0.0589	3.7490	**0.0002**
zsd	0.3385	0.0506	6.6940	**<0.0001**	0.3129	0.0594	5.2710	**<0.0001**
zcv	0.1827	0.0512	3.5650	**<0.0001**	0.1822	0.0562	3.2400	0.0012
pzabovezmean	-0.1181	0.0483	-2.4440	0.0145	-0.2537	0.0580	-4.3760	**<0.0001**
pzabove0.5	0.2039	0.0497	4.1070	**<0.0001**	0.1959	0.0574	3.4140	**0.0006**
zq10	0.0630	0.0493	1.2790	0.2010	0.0315	0.0566	0.5560	0.5780
zq20	0.0976	0.0494	1.9750	0.0483	-0.0026	0.0563	-0.0460	0.9630
zq30	0.1276	0.0478	2.6660	0.0077	0.0607	0.0593	1.0230	0.3060
zq40	0.1612	0.0482	3.3480	**<0.0001**	0.1638	0.0599	2.7330	0.0063
zq50	0.2097	0.0483	4.3390	**<0.0001**	0.1974	0.0604	3.2680	0.0011
zq60	0.2388	0.0486	4.9150	**<0.0001**	0.2037	0.0601	3.3910	**0.0007**
zq70	0.2402	0.0488	4.9170	**<0.0001**	0.2257	0.0596	3.7900	**0.0002**
zq80	0.2615	0.0492	5.3130	**<0.0001**	0.2621	0.0595	4.4030	**<0.0001**
zq90	0.2962	0.0500	5.9240	**<0.0001**	0.2764	0.0585	4.7220	**<0.0001**

Table A2. 16 m_2 Resolution Correlation Matrix of LiDAR variables. Values are correlation coefficients (r).

LiDAR Variables	zmax	zmean	zsd	pzabovezmean	pzabove0.5	zq10	zq20	zq30	zq40	zq50	zq60	zq70	zq80	zq90	zcv
	zmax	zmean	zsd	pzabovezmean	pzabove0.5	zq10	zq20	zq30	zq40	zq50	zq60	zq70	zq80	zq90	zcv
zmax	1.00	0.73	0.89	0.07	0.72	0.01	0.04	0.17	0.37	0.49	0.58	0.66	0.72	0.80	-0.06
zmean	0.73	1.00	0.91	0.65	0.91	0.08	0.16	0.40	0.72	0.86	0.94	0.97	0.96	0.93	-0.46
zsd	0.89	0.91	1.00	0.41	0.86	0.00	0.04	0.19	0.48	0.64	0.76	0.87	0.92	0.97	-0.40
pzabovezmean	0.07	0.65	0.41	1.00	0.60	0.05	0.13	0.32	0.54	0.65	0.69	0.68	0.64	0.54	-0.69
pzabove0.5	0.72	0.91	0.86	0.60	1.00	0.09	0.20	0.39	0.60	0.73	0.82	0.87	0.90	0.89	-0.50
zq10	0.01	0.08	0.00	0.05	0.09	1.00	0.59	0.21	0.10	0.07	0.06	0.05	0.04	0.03	-0.03
zq20	0.04	0.16	0.04	0.13	0.20	0.59	1.00	0.46	0.22	0.16	0.14	0.11	0.10	0.08	-0.07
zq30	0.17	0.40	0.19	0.32	0.39	0.21	0.46	1.00	0.56	0.43	0.36	0.31	0.28	0.25	-0.16
zq40	0.37	0.72	0.48	0.54	0.60	0.10	0.22	0.56	1.00	0.84	0.73	0.64	0.57	0.51	-0.26
zq50	0.49	0.86	0.64	0.65	0.73	0.07	0.16	0.43	0.84	1.00	0.91	0.81	0.74	0.66	-0.34
zq60	0.58	0.94	0.76	0.69	0.82	0.06	0.14	0.36	0.73	0.91	1.00	0.93	0.86	0.78	-0.40
zq70	0.66	0.97	0.87	0.68	0.87	0.05	0.11	0.31	0.64	0.81	0.93	1.00	0.96	0.88	-0.45
zq80	0.72	0.96	0.92	0.64	0.90	0.04	0.10	0.28	0.57	0.74	0.86	0.96	1.00	0.95	-0.48
zq90	0.80	0.93	0.97	0.54	0.89	0.03	0.08	0.25	0.51	0.66	0.78	0.88	0.95	1.00	-0.49
zcv	0.06	-0.46	-0.40	-0.69	-0.50	-0.03	-0.07	-0.16	-0.26	-0.34	-0.40	-0.45	-0.48	-0.49	1.00

Table A3. 250 m_2 Resolution Correlation Matrix of LiDAR variables. Values are correlation coefficients (r).

LiDAR Variables	zmax	zmean	zsd	pzabovezmean	pzabove0.5	zq10	zq20	zq30	zq40	zq50	zq60	zq70	zq80	zq90	zcv
	zmax	zmean	zsd	pzabovezmean	pzabove0.5	zq10	zq20	zq30	zq40	zq50	zq60	zq70	zq80	zq90	zcv
zmax	1.00	0.86	0.96	0.36	0.80	0.25	0.38	0.51	0.64	0.74	0.80	0.85	0.89	0.94	-0.18
zmean	0.86	1.00	0.84	0.65	0.90	0.46	0.64	0.78	0.89	0.94	0.96	0.97	0.96	0.93	-0.46
zsd	0.96	0.84	1.00	0.42	0.76	0.09	0.23	0.39	0.58	0.72	0.81	0.87	0.92	0.96	-0.28
pzabovezmean	0.36	0.65	0.42	1.00	0.61	0.25	0.41	0.55	0.65	0.68	0.69	0.67	0.63	0.55	-0.81
pzabove0.5	0.80	0.90	0.76	0.61	1.00	0.43	0.58	0.69	0.77	0.82	0.85	0.86	0.87	0.86	-0.47
zq10	0.25	0.46	0.09	0.25	0.43	1.00	0.75	0.58	0.47	0.40	0.37	0.33	0.31	0.28	-0.23
zq20	0.38	0.64	0.23	0.41	0.58	0.75	1.00	0.83	0.68	0.59	0.54	0.50	0.47	0.43	-0.30
zq30	0.51	0.78	0.39	0.55	0.69	0.58	0.83	1.00	0.86	0.76	0.70	0.65	0.61	0.57	-0.36
zq40	0.64	0.89	0.58	0.65	0.77	0.47	0.68	0.86	1.00	0.91	0.85	0.80	0.76	0.71	-0.41
zq50	0.74	0.94	0.72	0.68	0.82	0.40	0.59	0.76	0.91	1.00	0.95	0.91	0.86	0.81	-0.44
zq60	0.80	0.96	0.81	0.69	0.85	0.37	0.54	0.70	0.85	0.95	1.00	0.97	0.93	0.88	-0.46
zq70	0.85	0.97	0.87	0.67	0.86	0.33	0.50	0.65	0.80	0.91	0.97	1.00	0.97	0.93	-0.46
zq80	0.89	0.96	0.92	0.63	0.87	0.31	0.47	0.61	0.76	0.86	0.93	0.97	1.00	0.97	-0.46
zq90	0.94	0.93	0.96	0.55	0.86	0.28	0.43	0.57	0.71	0.81	0.88	0.93	0.97	1.00	-0.42
zcv	-0.18	-0.46	-0.28	-0.81	-0.47	-0.23	-0.30	-0.36	-0.41	-0.44	-0.46	-0.46	-0.46	-0.42	1.00

Table A4. Results from the final resource selection function (RSF) models, estimating probability of use as a function of LiDAR variables at resolutions 250m$_2$ and 16m$_2$. RSFs were generalized mixed models with binary response variable (Y = 1 = used, Y = 0 = available) and logit link function, assuming binomial error distribution. Input data for used and available observations were tracking locations of individuals bats, for which bats were within 25 m of the observer (Y=1) and an equal number of random observations (Y = 0) generated within the 95% minimum convex polygon home range for each individual bat. Bat ID nested in species was included as random effects to account for repeated observations of the same individuals.

LiDAR variable	β	SE	z	p
250 m$_2$				
Intercept (*M. brandtii*)	0.056	0.05873	0.951	0.342
M. mystacinus	-0.108	0.08118	-1.334	0.182
Maximum height (zmax)	0.562	0.05021	11.198	<0.0001
60th quantile of height (zq60)	-0.062	0.07798	-0.798	0.425
Coefficient of variation in height (zcv)	0.248	0.05863	4.228	<0.0001
Species × zq60	0.265	0.08975	2.956	0.003
Species × zcv	0.385	0.10030	3.840	<0.001
Random effects: Bat ID nested in Species: σ = 0.0007192, SD = 0.02682, Species: σ <0.000001, SD <0.000001				
16 m$_2$				
Intercept (*M. brandtii*)	0.044	0.056	0.781	0.435
M. mystacinus	-0.092	0.077	-1.200	0.230
Maximum height (zmax)	0.393	0.039	9.995	<0.0001
Coefficient of variation in height (zcv)	0.264	0.040	6.533	<0.0001
Random effects: Bat ID nested in Species: σ = 0.00, SD = 0.00, Species: σ <0.000001, SD <0.000001				

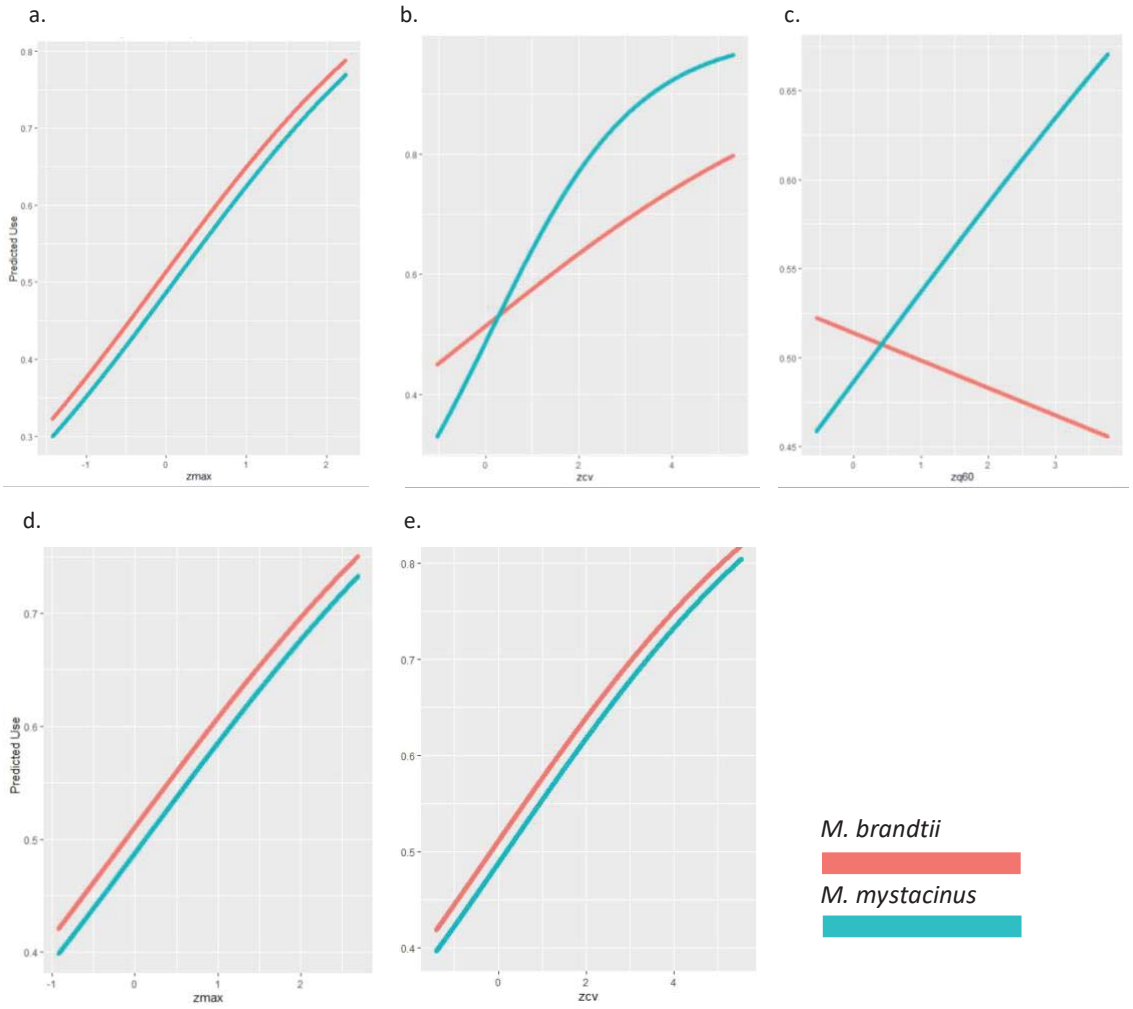

Figure A1. Predicted habitat use as a function of LiDAR variables. Lines show predicted relationships for each of the LiDAR variables in the final models in Table A4, at average values of the other LiDAR variables in the model. Red and blue lines represent *M. brandtii* and *M. mystacinus* respectively. Figures A1.a, b. and c. are results from the 250 m_2 resolution RSF model and Figures A1.d and e. are results from the 16 m_2 resolution RSF.

A2: Genetics - Additional Resources

Table A5. Sample orientation and primer combinations for each sample. Dual indexing of each sample allows accurate identification of samples after sequencing using bioinformatics tools. Some samples were run in duplicates to verify the accuracy of the barcoding method (e.g. 2, 8, 12, etc.). “Positive” indicates a DNA sample that was extracted from a known butterfly species.

		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
		i7_N701	i7_N702	i7_N703	i7_N704	i7_N705	i7_N706	i7_N707	i7_N710	i7_N711	i7_N712	i7_N714	i7_N715
A	i5_S502	positive	12	21	33	44	58	66	20	42	63	15	50
B	i5_S503	1	13	22	34	46	59	67	28	43	64	25	51
C	i5_S505	2	14	23	36	47	60	71	32	48	66	30	52
D	i5_S506	3	16	24	37	48	61	72	34	54	72	31	53
E	i5_S507	4	18	26	38	49	62	2	35	56	6	35	68
F	i5_S508	5	17	27	40	54	63	8	36	57	7	39	69
G	i5_S510	8	19	28	42	56	64	12	38	59	10	41	70
H	i5_S511	9	20	32	43	57	65	13	40	61	11	45	73

Table A6. Describes the names and genetic sequences of primers used in the indexing PCR.

Primer name	primer sequence
NGS_i5_S502	5'-AAT GAT ACG GCG ACC ACC GAG ATC TAC AC CTCTCTAT TC GTC GGC AGC GTC-3'
NGS_i5_S503	5'-AAT GAT ACG GCG ACC ACC GAG ATC TAC AC TATCCTCT TC GTC GGC AGC GTC-3'
NGS_i5_S505	5'-AAT GAT ACG GCG ACC ACC GAG ATC TAC AC GTAAGGAG TC GTC GGC AGC GTC-3'
NGS_i5_S506	5'-AAT GAT ACG GCG ACC ACC GAG ATC TAC AC ACTGCATA TC GTC GGC AGC GTC-3'
NGS_i5_S507	5'-AAT GAT ACG GCG ACC ACC GAG ATC TAC AC AAGGAGTA TC GTC GGC AGC GTC-3'
NGS_i5_S508	5'-AAT GAT ACG GCG ACC ACC GAG ATC TAC AC CTAAGCCT TC GTC GGC AGC GTC-3'
NGS_i5_S510	5'-AAT GAT ACG GCG ACC ACC GAG ATC TAC AC CGTCTAAT TC GTC GGC AGC GTC-3'
NGS_i5_S511	5'-AAT GAT ACG GCG ACC ACC GAG ATC TAC AC TCTCTCCG TC GTC GGC AGC GTC-3'
NGS_i7_N701	5'-CAA GCA GAA GAC GGC ATA CGA GAT TCG CCT TA GTC TCG TGG GCT CGG-3'
NGS_i7_N702	5'-CAA GCA GAA GAC GGC ATA CGA GAT CTAGTACG GTC TCG TGG GCT CGG-3'
NGS_i7_N703	5'-CAA GCA GAA GAC GGC ATA CGA GAT TTCTGCCT GTC TCG TGG GCT CGG-3'
NGS_i7_N704	5'-CAA GCA GAA GAC GGC ATA CGA GAT GCTCAGGA GTC TCG TGG GCT CGG-3'
NGS_i7_N705	5'-CAA GCA GAA GAC GGC ATA CGA GAT AGGAGTCC GTC TCG TGG GCT CGG-3'
NGS_i7_N706	5'-CAA GCA GAA GAC GGC ATA CGA GAT CATGCCTA GTC TCG TGG GCT CGG-3'
NGS_i7_N707	5'-CAA GCA GAA GAC GGC ATA CGA GAT GTAGAGAG GTC TCG TGG GCT CGG-3'
NGS_i7_N710	5'-CAA GCA GAA GAC GGC ATA CGA GAT CAGCCTCG GTC TCG TGG GCT CGG-3'
NGS_i7_N711	5'-CAA GCA GAA GAC GGC ATA CGA GAT TGCCTCTT GTC TCG TGG GCT CGG-3'
NGS_i7_N712	5'-CAA GCA GAA GAC GGC ATA CGA GAT TCCTCTAC GTC TCG TGG GCT CGG-3'
NGS_i7_N714	5'-CAA GCA GAA GAC GGC ATA CGA GAT TCATGAGC GTC TCG TGG GCT CGG-3'
NGS_i7_N715	5'-CAA GCA GAA GAC GGC ATA CGA GAT CCTGAGAT GTC TCG TGG GCT CGG-3'

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